HISTOLOGICAL AND CYTOLOGICAL STUDIES OF MURINE LEPROSY *

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Histological studies of localized lesions developing at the site of inoculation in the various species of animals employed have been invaluable in determining whether or not true infection, in the sense of a progressive multiplication of the organisms introduced, had taken place. Animals showing generalized infection furnished excellent material for the study of the origin and development of the cells (lepra cells) which act as hosts for the lepra bacilli in various organs and tissues. The study of early lesions is particularly important for this purpose.

In rats the infection tends, for a time, to remain localized at the site of inoculation, but in all instances distant organs were eventually involved. This metastatic involvement occurs in an irregular manner, but sooner or later, if the animal lives long enough, nearly every organ and tissue may be massively involved. As has been brought out above, massive infection by direct injection into an organ usually resulted in the rapid development of extensive lesions there. The kidney was comparatively resistant to infection perhaps because this organ contains fewer mesenchymal cells of the type that readily become infected. No histological or cytological differences were observed between the local and metastatic lesions produced in mice and those produced in rats. Certain peculiarities of the lesions developing in monkeys and in rabbits will be brought out later.

In general, the pathological changes are closely parallel to those in human leprosy. Lepra cells, the cytoplasm of which is densely packed and often distended with bacilli, increase at the expense of normal tissues which undergo extensive pressure atrophy. Cell nuclei, which are never invaded, are usually normal in appearance,

* This work was aided by a generous grant from the American Academy of Arts and Sciences.

Received for publication March 25, 1938.

but occasionally are compressed and pyknotic. They are often flattened along the periphery of the cell, giving a "signet ring" appearance like that of the normal fat cell. The vacuolated or foamy appearance of the cytoplasm of infected cells, which is characteristic of the human lesion, has only very rarely been observed in our material. Giant cells are often present in large numbers.

The observations made are in harmony with the belief that murine lepra bacilli are capable of multiplying only within cells. Extensive areas of cell degeneration within lepra nodules, resulting probably from infarction, are frequently seen. In such areas extracellular bacilli set free by the dissolution of their host cells persist in enormous numbers apparently because of their great resistance to autolysis and heterolysis. Such bacilli are often disseminated in tissues during the process of preparing the sections. In no instance has the distribution of organisms appeared to justify the assumption of extracellular multiplication.

Lepra bacilli are commonly believed to be capable of growing only in mesenchymal cells of a certain type. Various workers, including Oliver,[1] Henderson,[2] and Lowe,[3] have concluded that the lepra cells are derived from the cells that constitute the reticuloendothelial system. This concept, with certain exceptions that will be noted below, is in general supported by the present studies. In taking up lepra bacilli the cells of the reticuloendothelial system are merely carrying out one of their normal functions, the phagocytosis of lipoid structures that are physiologically useless or detrimental to the organism. It is the ability of the ingested bacilli to multiply within these cells, instead of being metabolized there, that makes possible the development of progressive and eventually fatal lesions. As the lesion progresses new lepra cells are formed, not only from the local mesenchymal cells, but also by mitotic division of preëxisting lepra cells, so that the process is in some respects analogous to a neoplasm. Lepra cells are found in mitosis with sufficient frequency to suggest that the organisms, or some product of their metabolism, stimulate cell division. The lepra cells do not, however, acquire any of the morphological characteristics of malignant tumor cells.

Mesenchymal Tissues: Cells that are commonly considered to be members of the reticuloendothelial system, such as the Kupffer

cells of the liver and the cells lining sinuses and forming the reticulum of spleen, lymph nodes and bone marrow, were found, by a study of the earliest lesions, to be initially infected. Isolated Kupffer cells, for example, were often infected (Fig. 3) and frequently distended with bacilli. Ordinary vascular endothelium, including that of arterioles, venules and precapillaries, was never found infected and was almost invariably in a flattened quiescent state, even when these vessels were within nodules composed almost entirely of lepra cells. Smooth, striated and cardiac muscle fibers likewise appeared invariably to escape infection, although often studied in locations where they must have been adequately exposed. The same statement may be made regarding cartilage cells and also serosal and meningeal lining cells. The observations regarding the latter two types of cell are particularly valid, because these cells were very frequently seen as single rows of flattened, uninfected cells overlying tissue composed almost entirely of lepra cells. Polymorphonuclear leukocytes, which were present in the lesions in relatively small numbers, often contained bacilli but never in such numbers as to suggest multiplication within these cells. Cells that could be recognized as lymphocytes, plasma cells and eosinophiles were always free from infection. It will be noted that the cells thus far described which were found infected are precisely those commonly considered as members of the reticuloendothelial system on the basis of their reaction to intravenously injected finely divided foreign substances.

The question of the reaction of fibroblasts and fat cells to infection could not be answered definitely by these studies. Large areas of ordinary connective and adipose tissue often became replaced by tissue which appears to be composed entirely of lepra cells, with a network of thin walled blood vessels. It seems probable that both the fibroblast and the lipoblast may eventually become infected, but one cannot rule out the possibility that they may disappear by pressure atrophy at the expense of lepra cells originating from histiocytes and by mitotic division of preëxisting lepra cells. The mesenchymal cells immediately adjacent to the sarcolemma of striated muscle fibers, regarded by some workers as specialized cells responsible for the formation of sarcolemma, become infected very early (Fig. 6).

In early lesions involving the testis isolated groups of interstitial

cells were often found infected (Fig. 2). This observation is in harmony with the view that certain of these interstitial cells belong to the reticuloendothelial system. In late lesions the interstitial cells appeared to become uniformly infected; here again, perhaps, as a result of pressure atrophy of those cells that were not originally invaded. After intracerebral inoculation infection commonly spread in the subarachnoid tissue over the entire surface of the brain, reaching the interior of the brain by following along the walls of the blood vessels (Fig. 1). In several instances infection also spread in the subarachnoid tissue of the spinal cord and produced extensive lesions of the cauda equina and peripheral nerves. Atrophy of the nerves was evidently due to the conversion of the mesenchymal cells into lepra cells and not to actual invasion of the nerve fibers. Ganglion cells were not found infected.

In the kidney, where infection was infrequent and rarely progressed to a point where lesions were grossly visible, the cells initially infected were mesenchymal cells in glomeruli and in the walls of intertubular capillaries and larger vessels. In the adrenal isolated mesenchymal cells or small clusters of mesenchymal cells were frequently infected. Such cells were found in both cortex and medulla but were, in several instances, most numerous at the junction of cortex and medulla. In bone marrow both extravascular and intravascular mesenchymal cells appear to be initially infected. Eventually by the proliferation of such cells the marrow becomes entirely replaced by lepra cells, the marrow spaces become greatly enlarged, and there is marked atrophy and disappearance of bony trabeculi (Fig. 7).

Epithelium: In general, it may be said that epithelial cells of all types were relatively resistant to infection. Liver cells, pancreatic acinar and islet cells, gastro-intestinal epithelium, and mucous gland and bronchial epithelium were invariably found to be free from infection, even when embedded in nodular masses of heavily infected mesenchymal cells.

In rare instances ependymal cells and neuroglia cells appeared to be lightly but definitely infected. In 1 rat lepra bacilli were found within epithelial cells of the epidermis in such large numbers as to indicate that intracellular multiplication had taken place. This rat was inoculated intracerebrally and extensive metastases

developed in the cervical lymph nodes. Cells forming all three layers of the epidermis overlying these nodes were infected, and localized multiplication of these infected cells had obviously taken place, sometimes producing small raised nodules with hyperkeratinization and sometimes causing localized areas of downward growth into the corium. In 1 mouse, also, the epithelial cells lining the tubules of the testes (Fig. 2) and the ducts of the epididymis were extensively invaded, so that the infected structures could easily be selected with the low power lens. This mouse was injected in the spleen with a suspension of lepra bacilli and showed extensive secondary involvement of the inguinal nodes and testis.

These observations were so infrequent that they should be considered as exceptions rather than the rule. Their chief importance lies in their suggestion that the relative resistance of epithelial cells to infection depends on their feeble phagocytic powers or on the inability of the bacilli to enter them, rather than upon intracellular conditions that are inhibitory to the growth of lepra bacilli.

Marchoux and Sorel [4] in 1912 studied the skin lesions of murine leprosy and noted in certain areas the presence of lepra bacilli in epithelial cells, an observation that has not attracted the attention of subsequent workers.

Lesions Produced in Monkeys and Rabbits

Although definite evidence of progressive local and metastatic infection of rat leprosy in both monkeys and rabbits was obtained there is reason to believe that *B. leprae murium* grows less readily within the cells of these species than in those of rats and mice. In general, infected cells contained fewer organisms and infected tissues, stained by the Ziehl-Neelsen method, were never definitely red in color when examined with the unaided eye, as they commonly were in rats and in mice. Although numerous miliary lepromas were found in the livers of monkeys after intracerebral inoculation, lepra bacilli were scarce in such lesions and were found only after prolonged research. The cells composing these lepromas also differed from the lepra cells seen in rats and mice in that they showed the cytoplasmic vacuolization which is characteristic of the human lepra cell.

Lesions Produced by Intracerebral Injection of Other Acid-Fast Organisms

Mice surviving the intracerebral injection of non-pathogenic acid-fast organisms never showed metastatic lesions. The organisms introduced called out many macrophages and neutrophiles. Organisms were found in large extracellular masses, but they were also taken up in large numbers by both macrophages and neutrophiles. A few days after injection these organisms showed evidence of degeneration, and after about 3 weeks they had entirely disappeared, although the inflammatory reaction was undiminished in intensity. No evidence of multiplication of these non-pathogenic organisms in the tissues was obtained, and the lesions produced by them could be readily distinguished from those of leprosy.

Avirulent tubercle bacilli (BCG) persisted longer in the brain, and several weeks after injection were found in large numbers in macrophages, presenting a picture more closely resembling that of leprosy. Organisms never distended the cells greatly, however. It was not possible to determine whether or not multiplication of these organisms had occurred in the tissues, but metastatic lesions did not develop.

With the virulent strain of the tubercle bacillus (H 37) lesions were produced in mice which simulated even more closely those of leprosy and which were accompanied by extensive metastatic lesions in the lungs (Fig. 8) and spleen. In these metastatic lesions organisms were present in enormous numbers and were largely within macrophages. These tuberculous lesions were not caseous and were often practically identical with those of leprosy, although the tubercle bacilli themselves could be identified because of their larger size and morphological characteristics. These metastatic tuberculous lesions developed only after intracerebral inoculation; subcutaneous inoculation of similar doses did not result in localized or metastatic infection.

Summary

Lepra cells in murine leprosy are derived largely from mesenchymal cells belonging to the reticuloendothelial system. Exceptionally, however, epithelial cells, specifically those of the epidermis, testicular tubules and epididymis, became distended with

lepra bacilli. This observation suggests that the relative resistance of epithelial cells to infection may depend on the inability of lepra bacilli to enter them, rather than on intracellular conditions unfavorable to their growth.

In infected rats and mice surviving for a long time the tissues of practically all organs were extensively replaced by non-vacuolated lepra cells distended with bacilli, but kidney tissue contained relatively few of these cells.

In the progressive local and metastatic lesions produced with *B. leprae murium* in rabbits and monkeys lepra cells were often vacuolated and acid-fast bacilli were much less numerous in these lesions than in those produced in rats and mice, and were found only after prolonged search.

Non-pathogenic acid-fast bacilli, injected intracerebrally, were taken up by macrophages and neutrophiles, but disappeared from the lesions in a few weeks, never producing metastatic lesions.

Virulent tubercle bacilli, although innocuous when injected subcutaneously into mice, produced progressive and metastatic infection when injected intracerebrally into these animals. The lesions were non-caseating and the tubercle bacilli were found largely within macrophages, so that these lesions closely resembled those of leprosy.

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DESCRIPTION OF PLATE

All sections were prepared from tissues fixed in 10 per cent formalin in 95 per cent alcohol and stained by the Ziehl-Neelsen method.

PLATE 122

FIG. 1. Cerebral cortex of mouse after intracerebral injection of murine lepra bacilli showing phagocytic cells laden with acid-fast bacilli filling the perivascular space. The vascular endothelium is not infected. × 570.

FIG. 2. Mouse testis after intrasplenic injection of murine lepra bacilli. Note infection of tubular epithelial cells and patchy distribution of lepra cells in the interstitial tissue. × 400.

FIG. 3. Liver of mouse after intracerebral injection of murine lepra bacilli showing infection of isolated Kupffer cells. × 1300.

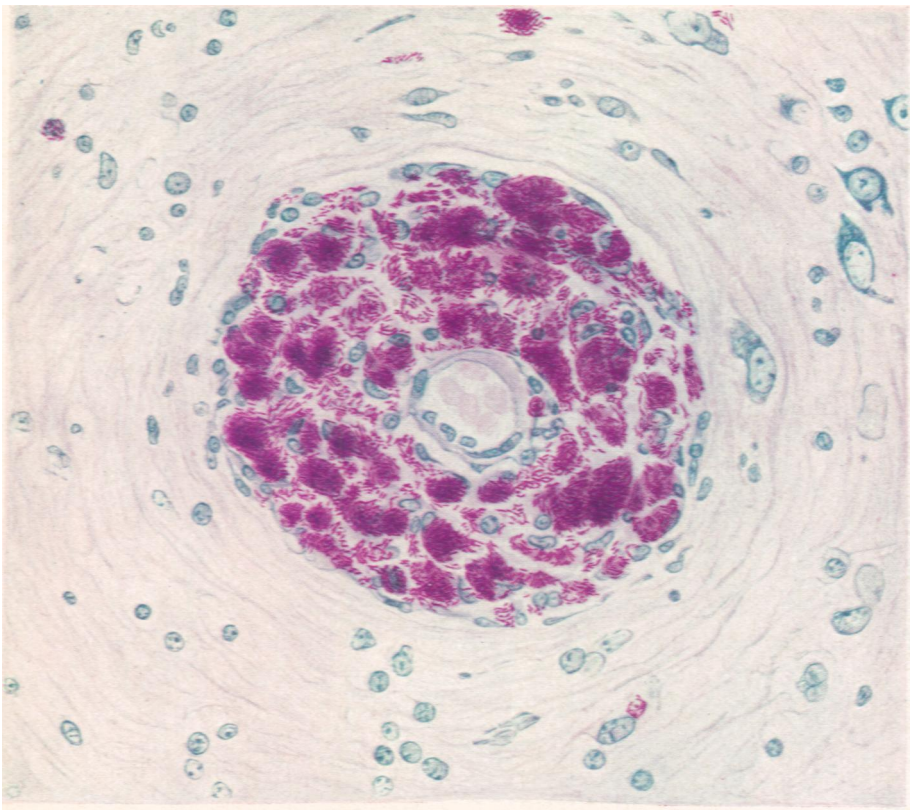
FIG. 4. Spinal nerve of rat after intracerebral injection of murine lepra bacilli. Lepra cells within the nerve bundle are derived from the mesenchymal cells there. The nerve fibers are not infected but disappear from pressure atrophy. × 570.

FIG. 5. Stomach of mouse after intracerebral injection of murine lepra bacilli showing lepra cells of mesenchymal origin. × 570.

FIG. 6. Striated muscle in rat leprosy showing infection of mesenchymal cells adjacent to the sarcolemma. × 570.

FIG. 7. Skull of rat after intracerebral injection of murine lepra bacilli showing accumulation of lepra cells in marrow space and consequent atrophy of bone trabeculae. × 570.

FIG. 8. Lung of mouse after intracerebral injection of virulent tubercle bacilli (H 37). The alveoli and alveolar walls contain many macrophages laden with tubercle bacilli, simulating the appearance of lepra cells. × 570.



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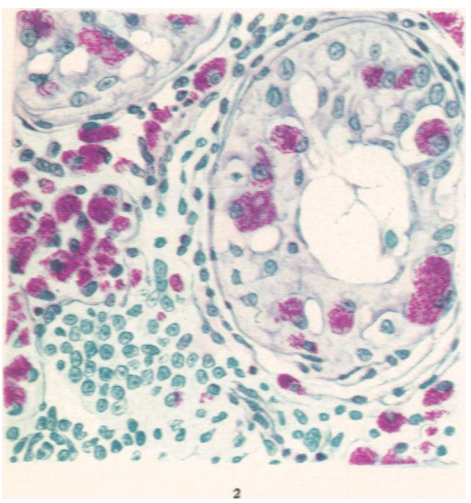

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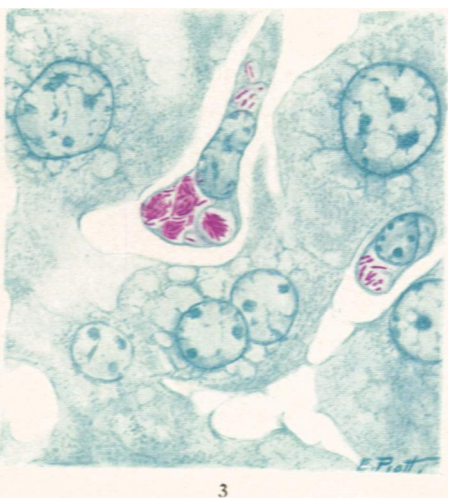

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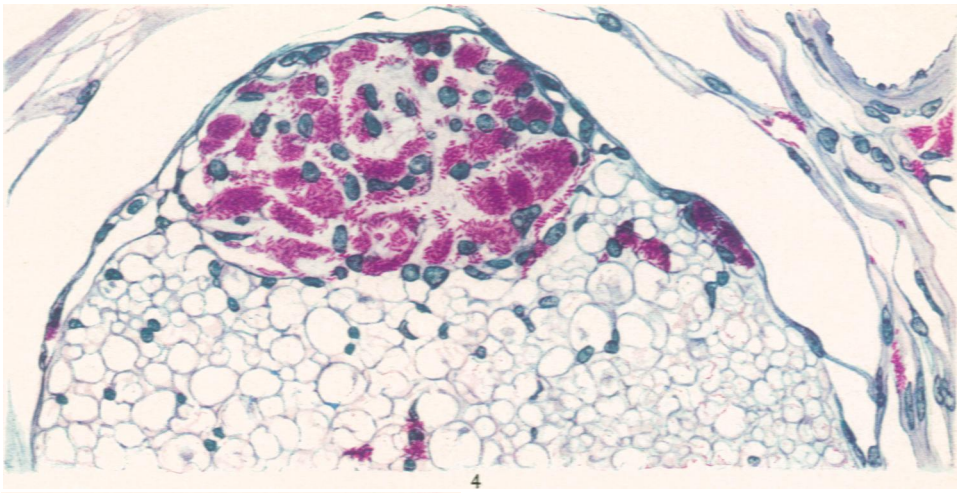

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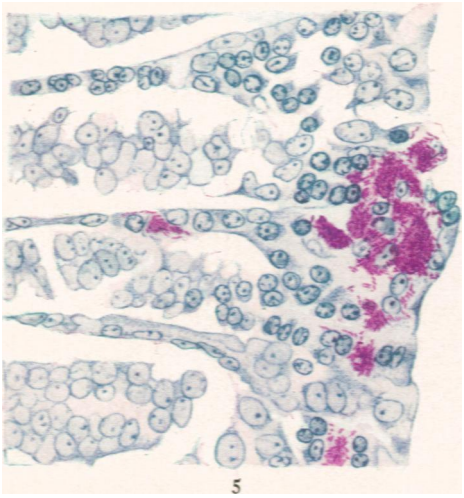

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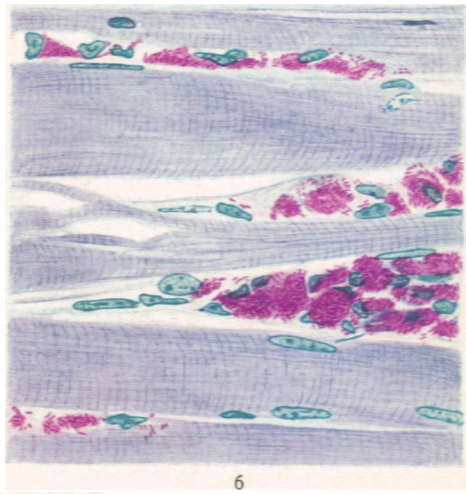

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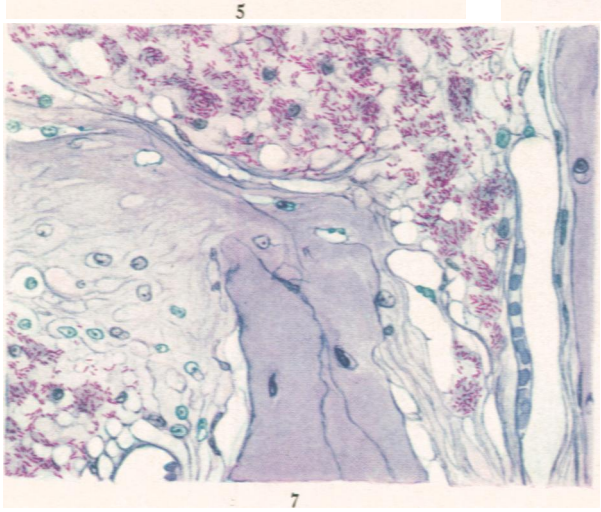

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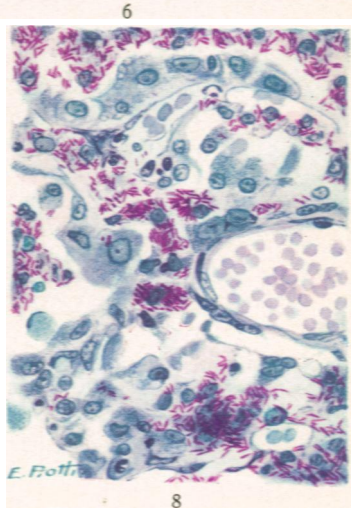

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Studies of Murine Leprosy