HISTOLOGICAL REACTIONS PRODUCED BY EXPERIMENTAL INOCULATION OF *MYCOBACTERIUM LEPRAE MURIUM* INTO THE GOLDEN HAMSTER (*CRICETUS AURATUS*)

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Experimental inoculation of *Mycobacterium leprae murium* into the golden hamster, first done by Balfour-Jones, produces a generalized infection of slow but progressive evolution, with lesions in various organs (2, 4, 14, 15). The Chinese hamster, however, is apparently not susceptible to this infection (13).

Observations on this infection of the hamster have been mainly from the bacteriological point of view. The pathology of the disease produced, and particularly the histological reactions, have seldom been considered; only some gross changes and a few notes about microscopic lesions have been published (14, 15). Nothing has been said about the cytogenesis, morphology, or functional behavior of the cells in the lesions, features which are of great value in defining the type of the tissue reaction produced by the mycobacteria (6, 7). The present study deals with some of the cytological characters of the lesions.

MATERIAL AND METHODS

Twenty adult golden hamsters of both sexes, weighing 125± 5gm., were injected intraperitoneally with 0.5 cc. of a suspension of triturated murine leprosy lesions. The approximate concentration of the bacilli in the suspension was determined after separation of the bacilli with chloroform (10). Quantities corresponding to about 6 mgm. of bacilli were inoculated.

The animals were weighed every week. One animal was killed with ether every 10 days until the 70th day after inoculation, and after that one every 20 days, until the end of the experiment. However, four hamsters died during the evolution of the infection, thus limiting the period of observation to 253 days.

Twenty rats, weighing 105± 5mgm., were injected by the same route and with the same quantity of bacilli. These animals served as controls for comparison with the inoculated hamsters, to be sure that the inoculum produced a progressive and fatal disease with all the characters of murine leprosy.

RESULTS

The dose of *M. leprae murium* injected did not seem to affect the general health of the hamsters, and they maintained approximately the same weight throughout the experiment. Natural deaths, however, oc-

curred 190 days after the inoculation. The maximum survival time was 253 days for the hamsters and 280 days for the control rats.

Macroscopic lesions.—The macroscopic lesions in the hamster were small until 150 days after inoculation, but from then on they became conspicuous. (1) The changes found in the earlier period were: inconstant and slight hypertrophy of the mediastinal and cervical lymph nodes; presence of pale areas on the liver surface, without appreciable modification of the volume of this organ; slight enlargement of the spleen, and areas of perisplenitis; and small thickened areas, adhesions, and sometimes a few abscess, in the peritoneal cavity. All of these lesions showed wide individual variations, and the total picture ranged from a complete absence of lesions on the one hand to the presence of clear-cut lesions in several organs on the other hand.

All the hamsters that were killed or that died were autopsied. For the histological studies the material was fixed in either 10 per cent formalin or an 80 per cent alcohol-formalin-acetic acid mixture (80:15:5). Frozen or paraffin sections were stained with hematoxylin and eosin, Masson's trichromic stain, the Ziehl-Neelsen method, methyl violet, toluidine blue, methyl green-pyronin, Sudan black B, Perls' method for iron, and by the periodic acid-Schiff (PAS) technique.

The lesions were not examined by bacteriological methods, because the hamster does not show spontaneous tuberculosis and very large quantities of bacilli are necessary to produce experimental tuberculosis (12).

(2) In the later period, after the 150th day, the lesions were similar to those of the earlier period but more pronounced. They affected mainly the spleen and the lymph nodes draining the peritoneal cavity, producing slight hypertrophy of those organs. The spleen showed some pale areas, which sometimes involved the greater part of the organ. Here again there were great individual variations in the degree of the lesions; only the spleen lesions were constant, and generally marked.

Microscopic lesions.—The microscopic lesions, of nodular shape, were most frequently found in organs rich in reticuloendothelial elements, particularly the spleen, lymph nodes, omentum and liver. They varied greatly in number and size. In the liver, the omentum, and eventually the lungs, they were initially located near the blood vessels and developed from the adventitial cells. In the spleen and lymph nodes, respectively, the reticular cells of the red pulp or of the cortex were the first to be affected. The initial phase of the lesions may be verified by the presence of bacilli within the phagocytic (macrophage) cells.

Abscesses sometimes found in the peritoneal cavity, the site of inoculation, had a central suppurative and necrotic area containing a few acid-fast bacilli and a fibrous capsule within which were bacillus-containing macrophages and large cells of xanthoma type (Fig. 1). These latter cells contained in their cytoplasm some PAS-positive or sudanophilic drop-

lets, and a few amorphous acid-fast granules. Cells of this type were sometimes seen in other organs, such as the lymph nodes and lungs.

Based on the cytological and bacteriological findings, the histological lesions can be classified into two types: (1) early lesions, found in the initial period of the evolution of the disease, until the sixth or seventh month; and (2) late lesions, observed during the final period of the disease, after the fifth month.

EARLY LESIONS

The early lesions contained few bacilli, which within ten days after the inoculation showed marked morphological alterations: fragmented and granular bacilli and acid-fast granules. As regards the cytological development of the lesions, the injected bacilli were phagocytized either by preexistent macrophages (reticular cells of the lymph nodes and spleen) or newly-developed macrophages. The latter arose from activated fixed cells, as in rat-leprosy lesions (6, 7), and proliferated to produce small groups. They showed vacuolated cytoplasm, and sometimes nuclear pyknosis. Necrosis of some macrophages was sometimes seen. These cells increased in number progressively, but the bacilli did not multiply at the same rate, so there was a relative reduction in their number (paucibacillary lesions).

Initially the macrophages had a network arrangement, as in rat-leprosy lesions, but after the tenth day they were more vacuolated and showed a more compact arrangement. At the same time the alterations of the bacilli became more pronounced. The lesions became nodular, like those of the rat, but there were morphological differences in the cells of which they were constituted. There was no proportionality between the scarce bacilli and the large number of cells, as there is in rat-leprosy lesions; and the cells contained variable quantities of an amorphous acid-fast material, morphologically different from bacillary granules, which probably arose from lysed bacilli.

That the cells which constitute these early lesions are macrophages is evidenced by their morphologic and functional character, not only by their phagocytic activity for bacilli but also by the presence of brown, iron-containing granules and sudanophilic and PAS-positive droplets. The lesions themselves were irregular in shape, did not become necrotic, and morphologically resembled the tuberculoid reactional lesions of human leprosy; but plasma cells predominated in the lymphoid infiltrate.

Sixty days after inoculation the lesions showed a few morphologically altered bacilli, and the involutive aspect—which was evident by the 10th day—was now more marked, the numbers of vacuolated cells being greater (Fig. 4). The lesions simulated those of murine leprosy in involution (5), differing from them by the rich plasma-cell infiltrate and by the presence of some neutrophils, and sometimes a small necrotic central zone.

Later on the involutive phenomena became still more marked, the cells

being dissociated by lymphoid and plasma cells, which preceded histological healing. In some cases, when the lesions had become free of bacilli or showed only a few acid-fast granules, the cells underwent a new morphologic transformation. They then showed a large and well-delimited acidophilic cytoplasm, which presented a hyalin or fibrillar appearance and was slightly PAS-positive and acid-resistant; generally these cells contained amorphous acid-fast droplets. The nucleus was small, eccentric, and showed pyknosis, this giving the cell the appearance of a plasma cell (Fig. 5). These cells had little activity in relation to hemosiderin as compared to the macrophages. These lesions sometimes showed giant cells and a lymphoid infiltrate with plasma cells predominant, assuming the tuberculoid aspect (Fig. 6). The constituent cells then had the morphologic characters of the epithelioid cell, but seldom contained acid-fast granules.

The involution of the lesions was slow but progressive; after 180 days histological healing of some of them might occur in various organs, through either fibrosis or hyalinization. The lesions generally disappeared without leaving a scar. During the initial period of evolution some organs showed macrophages, isolated or in small groups, containing numerous bacilli in the cytoplasm. This would indicate a constant spreading of bacilli, probably through the blood stream, a fact which seems to be related to the development of the late lesions.

LATE LESIONS

The cytogenesis of the late lesions, found after the 150th day following inoculation, was identical with that of the initial lesions: the macrophages, which had an apparent syncytial network arrangement, phagocytized the bacilli and proliferated, constituting lesions which at first were poorly delimited but which later became nodular (Figs. 8-14). The bacilli were numerous, and did not show the morphological alterations seen earlier. Lesions of this kind underwent rapid and progressive evolution, the bacilli increasing in numbers, the macrophages proliferating, and, with hypertrophy of the cytoplasm, becoming transformed to the hamster lepra cell.

This cell is initially oval and has a large, well-delimited, acidophilic cytoplasm; the nucleus is small, deeply-stained, and eccentrically placed, giving the cell a plasmacytoid aspect (plasmacytoid lepra cell, Fig. 11); these cells are separated one from another. Subsequently the cytoplasm, of granular or fibrillar structure, as well as sudanophilic droplets, increase in size and the cells become polyhedral (polyhedral lepra cell, Fig. 13); the structure is as dense as that of the lepra cell of rat leprosy. Both of these kinds of lepra cell are rich in bacilli (Fig. 14).

The late lesions did not show necrosis or peripheral lymphoid infiltration, and giant cells were rare. Sometimes asteroid bodies were found (Fig. 12), like those first observed by Wolbach (16). Late lesions could

be seen beside early lesions which still remained, sometimes even within the involutive initial lesions.

"Hyaline substance" lesions.—Besides these late lesions produced by the presence of the bacilli, another kind of lesion developed without bacilli. They were characterized by the deposition, mainly intracellularly, of a slightly acidophilic "hyalin substance." This substance did not stain metachromatically with methyl violet, but it did show metachromasia with toluidin blue and was slightly PAS positive. These lesions were found mainly in the spleen (Fig. 2), but also in the liver and lungs. In this latter organ the substance was localized in the septal cells, which afterwards fell into the alveolar spaces.

Lesions of this kind were seen as early as the 20th day after inoculation. They began by deposition of the hyalin substance in either the lepra cells of the involutive early lesions or the apparently healthy reticular cells. The affected cells showed hypertrophy of the cytoplasm; rarely the nucleus also underwent hypertrophy, assuming the appearance of the Sternberg cell. The reticular spleen-pulp cells were prematurely affected, the great cytoplasmic hypertrophy sometimes causing marked atrophy of the lymphatic follicles, apparently mechanically. In other organs, also, this process was responsible for compression phenomena; in the liver, retention of bile and degenerative alterations of the hepatic cells could be seen. In some animals, two months after the inoculation, these lesions became generalized and also reached the walls of some medium-sized blood vessels (Fig. 3). Four months after inoculation they underwent involution, disappearing slowly.

DISCUSSION

The inoculation of *M. leprae murium* into the peritoneal cavity of the hamster produces an infection of slow evolution which finally kills the animal. The disease thus produced evolves in two phases: (*a*) the initial phase, with early lesions of regressive character; and (*b*) the late phase, in which the lesions are progressive, rich in bacilli, and disturb vital functions of the organism.

The histological and bacteriological findings in these two phases are contradictory. The regressive character of the early lesions, which undergo spontaneous healing, might suggest that the hamster is not initially susceptible to this microorganism, but the rapidly progressive nature of the late lesions proves its susceptibility.

The genesis and morphology of the cells are identical in both kinds of lesions, and can be compared with those of the lepra cells of rat leprosy. Even the polyhedral lepra cell of the late lesions can be identified morphologically with some of the cells observed in the early lesions (Figs. 5 and 12). The main difference between the two types of lesions lies in the number and condition of the bacilli. Only the plasma-cell infiltration around the early lesions was not found in the late ones.

The paradoxical evolution of these two types of lesions in the hamster, which are related to the phase of the evolution of the disease, might be due to (*a*) differences in the functional behavior of the elements involved in the tissue reactions, or (*b*) failure of adaptation of the bacillus to the hamster's tissues.

Initially the bacilli are engulfed and destroyed by the macrophages, but, contrarily, during the final phase their uptake is followed by proliferation. The destruction in the early lesions seemed to be slow, yet the lesions soon became involutive. It might be concluded that at first the bacillus was unable to adapt itself, so that reduced proliferation together with the destruction that was seen were responsible for the paucibacillary lesions; but that later on there existed better adaptation, with abundant growth and consequently rapid evolution of the lesions. On the other hand, the observed facts might depend only upon modifications of the defense mechanism of the animal, with corresponding effects on the vitality of the infecting microorganism. In support of this alternative is the fact that serial transfer from hamster to hamster does not accelerate the evolution of the disease. That is always the same, i. e., a duration of 30 to 32 weeks, finally ending with death of the animal (2).

In the initial lesions, morphologically altered bacilli predominated. The alterations are identical to those observed in murine leprosy lesions of involutive character (5), but they appear earlier, the injected bacilli being more rapidly destroyed in the hamster than in the rat. Apparently the hamster macrophages are able to destroy the inoculated bacilli, although slowly, but it is difficult to determine the role of the macrophages in the bacterial lysis. For comparison, it has been observed (11) that in lesions produced by heat-killed *M. leprae murium* the bacilli undergo lysis more rapidly in the hamster than in the rat. On the other hand, this process in the hamster is much slower than in the guinea-pig under the same conditions (7, 8). It is possible that the hamster macrophages participate actively in the bacterial lysis, which would explain the presence of degenerative alterations and the large numbers of macrophages in the early lesions as compared to the small numbers of bacilli.

Identical facts were observed in the guinea-pig lesions, in which the bacilli seemed to be actively destroyed, in contradiction with the rat lesions where active destruction was not found (5). In the hamster's early lesions and in the guinea-pig tissues the stimulus exerted by the rat-leprosy bacillus on the macrophages seems to be stronger than a mechanical one. On the other hand, in the rat and in the late lesion of the hamster the bacilli seemed to stimulate the macrophages only mechanically.

The hamster's involutive lesions of tuberculoid aspect and without bacilli are constituted by cells which have some of the morphological and physiological characters of the guinea-pig epithelioid cell: slight phagocytosis and atrocytosis, and small quantities of lipids as compared with

the lepra cell. This fact suggests that these cells are active in the breakdown of the products liberated by the lysed bacilli. But the rate of lysis was slow, and the tuberculoid-like lesions without bacilli were observed in the hamster only 60 days after inoculation. On the other hand, the cells which constitute the early lesions are similar to, but not identical with, the epithelioid cells of the guinea-pig lesions.

To summarize, the early lesions of the hamster show spontaneous involution, which begins quickly and progresses slowly. These lesions have some peculiarities which permit distinguishing them from the involutive lesions of the rat. The cells of the hamster lesions have characteristics of both the guinea-pig epithelioid cell and the rat lepra cell. There are morphological and functional similarities between the cells of the early lesions of the hamster and those of the lesions of the tuberculoid reactional type of human leprosy.

On the other hand, the cells which constitute the late lesions of the hamster are morphologically and physiologically identical with cells of murine leprosy and human lepromatous leprosy. The hamster lepra cell has morphologic peculiarities proper to the animal species, but the similarity referred to is evident, especially if we have in mind their functional activity (atrocytosis, lipids, bacilli).

The finding of asteroid bodies in the late lesions was not related to the presence of bacilli. These bodies have also been found in tuberculous lesions in the hamster (1, 3).

The "hyalin substance" responsible for the secondary lesions described had certain peculiarities. It was found within reticular cells; it did not stain metachromatically with methyl violet, unlike amyloid; but it did stain metachromatically with toluidin blue and was slightly PAS positive, which means that it contained mucopolysaccharides. Its intracellular location suggests that the PAS-positive droplets, found mainly in the reticular cells of the spleen and in the lepra cells of the involutive lesions, are related to it. These droplets, however, do not stain metachromatically with toluidin blue.

SUMMARY

The intraperitoneal inoculation of about 6 mgm. of *M. leprae murium* into the golden hamster causes a slowly-developing disease which ends with the death of the animal. The disease during its evolution shows two phases: (1) an initial phase, with small macroscopic lesions histologically of retrogressive character, and (2) a late phase, observed after the 150th day after inoculation, with larger lesions which histologically are of progressive nature.

In the early lesions the bacilli are relatively few and show morphological alterations. This seems to be due to lysis of the bacilli in the tissue, probably with active participation of the macrophages. The bacilli seem not to multiply in the lesions, which undergo slow involution. The

late lesions, on the other hand, do not show lysis of the mycobacteria and they multiply actively.

The appearance and numbers of the bacilli in the lesions are related to the cells which constitute these lesions. In the initial lesions the cells have morphologic and physiologic characters which place them between the epithelioid cell of the guinea-pig on one hand, and the lepra cell of the rat on the other hand. In the late lesions, however, the cells have only the characters of rat lepra cell.

The differences between these two kinds of lesions seems to be due more to the tissue reaction of the hamster than to the adaptation of the mycobacteria to the animal's organism.

During the initial phase, secondary lesions occur in various organs caused by the deposition of a "hyalin substance." In the late phase, asteroid bodies may be seen in the lesions.

RESUMEN

La inoculación intraperitoneal de unos 6 mgm. de *M. leprae murium* en el "hamster" dorado (*Cricetus auratus*) provoca una enfermedad de desarrollo lento que culmina en la muerte del animal. Durante su evolución, la dolencia muestra dos fases: (1) fase inicial, con pequeñas lesiones microscópicas de naturaleza retrogresiva histológicamente y (2) fase tardía, observada a partir del 150° día de la inoculación, con lesiones más grandes que son histológicamente de naturaleza progresiva.

En las lesiones tempranas, los bacilos son relativamente escasos y muestran alteraciones morfológicas. Esto parece deberse a lisis de los bacilos en el tejido, probablemente con participación activa de los macrófagos. Los bacilos no parecen multiplicarse en las lesiones, que experimentan involución lenta. En cambio, las lesiones tardías no revelan lisis de las micobacterias y se multiplican activamente.

El aspecto y las cantidades de los bacilos de las lesiones se relacionan con las células que constituyen las últimas. En las lesiones iniciales, las células poseen caracteres morfológicos y fisiológicos que las colocan entre la célula epiteliodea del cobayo por un lado y la célula leprosa de la rata por otro. Sin embargo, en las lesiones tardías las células no poseen más que los caracteres de la célula de la lepra murina.

Las diferencias entre esas dos clases de lesiones parecen deberse más a la reacción histológica del hamster que a la adaptación de las micobacterias al organismo del animal.

Durante la fase inicial, ocurren lesiones secundarias en varios órganos, causadas por el depósito de una "substancia hialina." En la fase tardía, pueden observarse cuerpos asteroides en las lesiones.

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DESCRIPTION OF PLATES

All pictures and photomicrographs are of experimental lesions in the hamster, taken at 400x magnification. All sections were stained by hematoxylin and eosin except when otherwise indicated.

PLATE 7

FIG. 1. Peritoneal lesion, wall of abscess. Cells of xanthomatous type.

FIG. 2. Spleen. Early involutive lesion with numerous plasma cells (above), and an area with the "hyalin substance" deposited within the reticular cells of the red pulp.

FIG. 3. Liver. Hyalin substance deposited in the cells and blood-vessel walls of the portal space.

FIG. 4. Omentum. Involutive early lesion constituted by nodules which are separated by infiltrates rich in plasma cells.

FIG. 5. Omentum. Early lesion. On the right side, cells with morphologic characters of the hamster lepra cell, but containing only a few bacilli; on the left side, plasma and plasmacytoid cells, which latter do not contain bacilli and generally have a slightly PAS-positive cytoplasm.

FIG. 6. Omentum. Involutive early lesion of tuberculoid aspect.

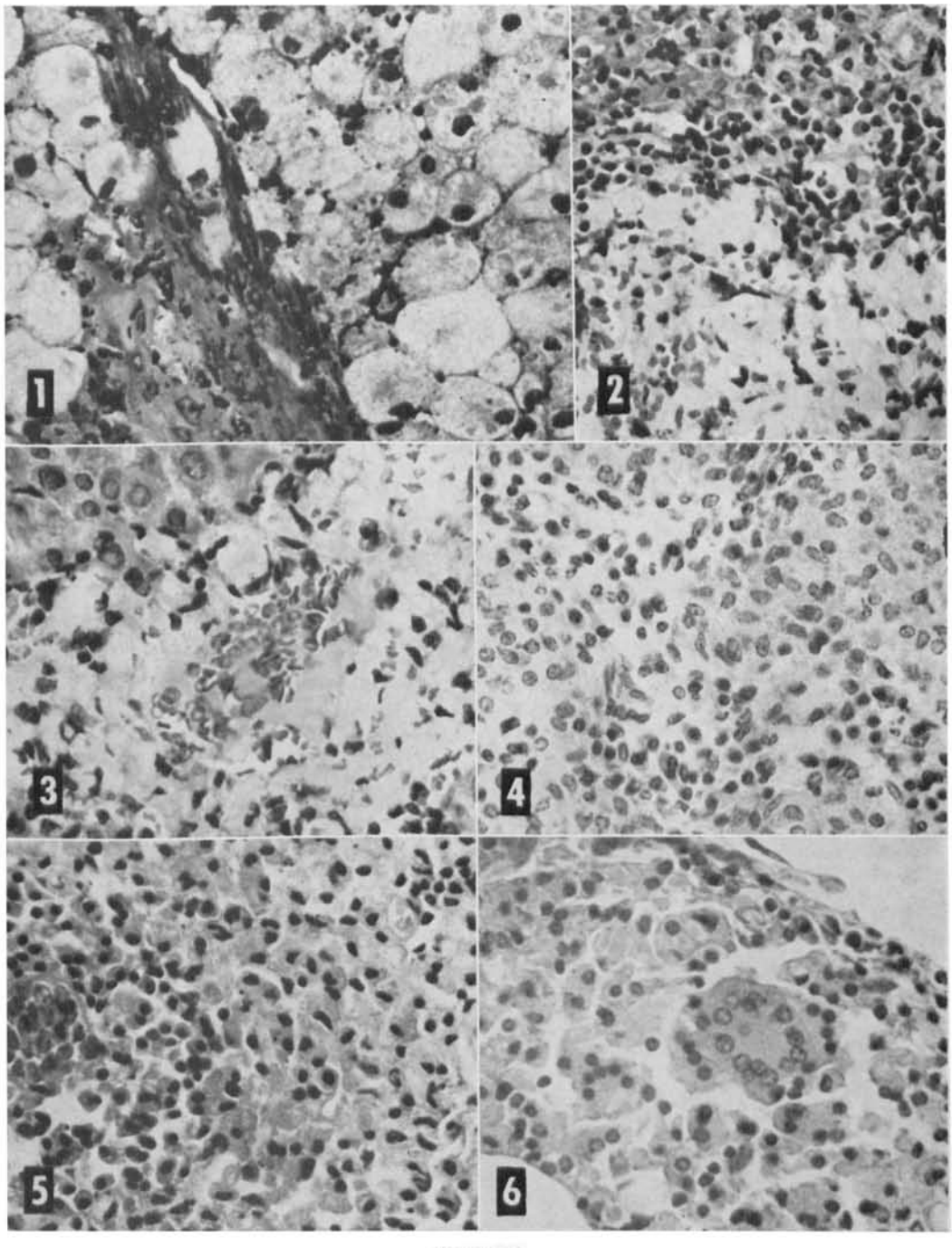

PLATE 7

PLATE 8

FIG. 7. Lymph node. An involutive early lesion which resembles the involutive rat lesion. In lesions of this type the cells show sudanophilic and PAS-positive droplets.

FIG. 8. Spleen. Late leprous lesion, constituted by a network of proliferating reticular cells.

FIG. 9. Lymph node. Late leprous lesion. Bacilli in the proliferating reticular cells. Ziehl-Neelsen.

FIG. 10. Lymph node. Late leprous lesion with plasmacytoid lepra cells. Cells of this type contain numerous bacilli.

FIG. 11. Omentum. Late leprous lesion, constituted by plasmacytoid lepra cells and one giant cell. Bacilli are numerous in this type of lesion.

FIG. 12. Lymph node. Late leprous lesion, with polyhedral lepra cells. Near the upper edge is an asteroid body.

FIG. 13. Spleen. Late leprous lesion with polyhedral lepra cells showing striated cytoplasm.

FIG. 14. Omentum. Bacilli in late leprous lesion. Ziehl-Neelsen.

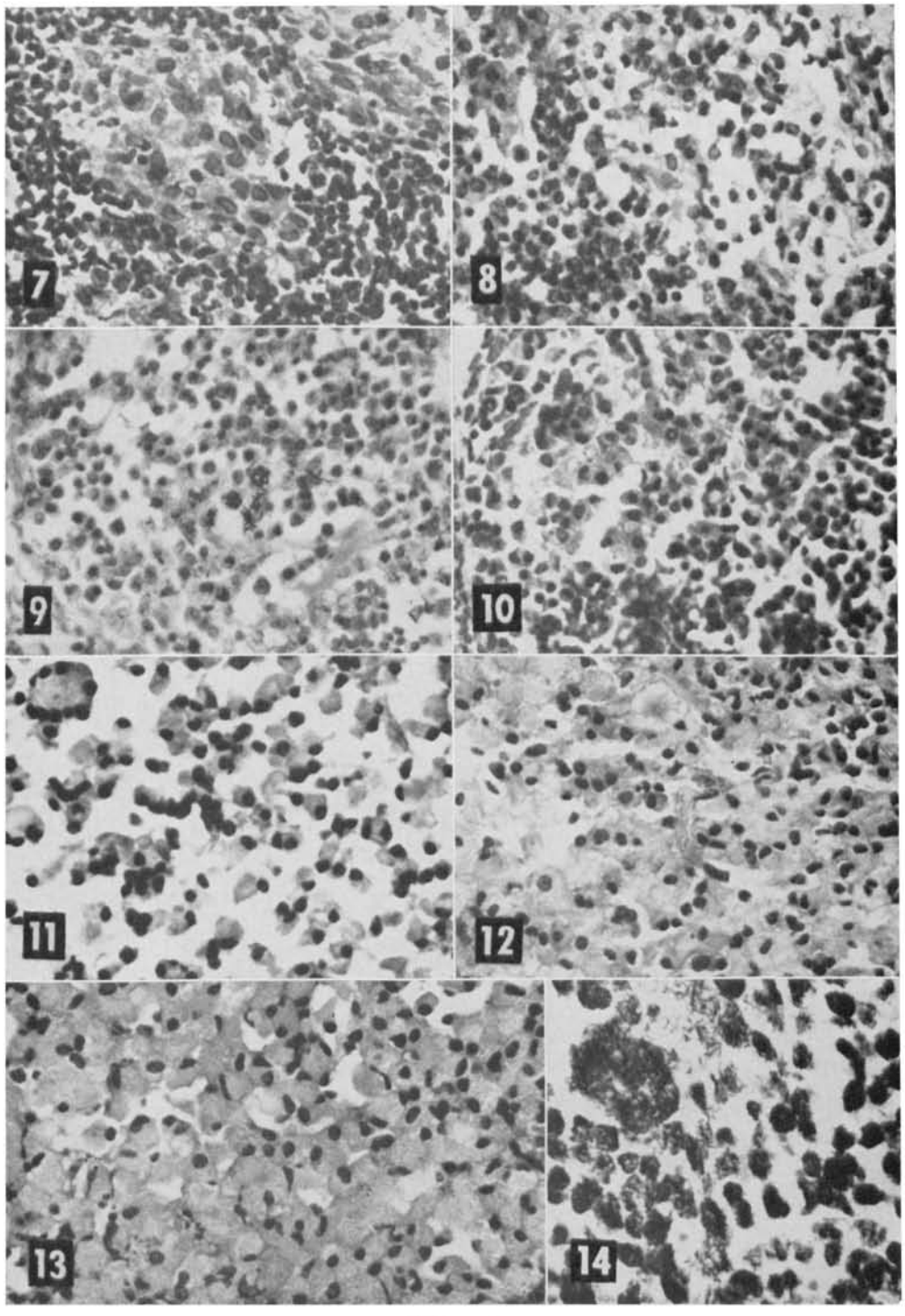

PLATE 8