# SILVERING OF LEPRA BACILLI IN TISSUES 1

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Although silver impregnations of tissues have been used previously (5) for the study of nerve changes in leprosy, and for the demonstration of reticulum, apparently silvering of the bacilli has not been observed. With the methods used by us, lepra bacilli have been found to be demonstrated routinely and effectively in biopsy material in more than 100 cases of leprosy.

#### PROCEDURES

The methods used have been those of Jahnel (2), Levaditi (3), and Manouélian (4), modified in the manner to be described. Jahnels' method is longer and more tedious, but it has been found particularly effective, giving beautiful results in tissues fixed in formaldehyde or Bouin's fluid for as long as one year. The modification employed is, briefly, as follows:

1. Fix tissue in 4 per cent formaldehyde solution or Bouin's fluid for two weeks or more.

2. Place in pyridine for one to three days.

3. Wash in several changes of distilled water for twenty-four hours.

4. Fix in 4 per cent formaldehyde for four days.

5. Wash in several changes of distilled water for twenty-four hours.

6. Place in 95 per cent alcohol, several changes, for three to eight days.

7. Wash in distilled water until pieces of tissue sink to the bottom of the container.

8. Treat with aqueous silver nitrate (0.5 per cent in distilled water) in the dark at 37 C. for five to eight days. A copious amount of the solution should be used.

9. Wash in distilled water ten minutes to remove excess silver.

10. Reduce for two days in a solution prepared as follows: pyrogallic acid, 4 gm.; 37 per cent formaldehyde solution, 5 cc.; distilled water, 95 cc.

11. Dehydrate, clear, embed, section and mount without further staining, by any routine method.

<sup>1</sup> From the Patronato para la Profilaxis de la Lepra y Sifilis, Havana, and the United States Marine Hospital, Carville. Reprinted verbatim, with permission, from the *Archives of Pathology* **46** (1948) 542-549, the original cuts having been loaned through the kindness of Dr. Morris Fishbein.

## 17,4 León Blanco, Fite: Silvering of Lepra Bacilli

## RESULTS

Lepra bacilli appear in various shades of black and brown, the majority in an intense black or blue-black. Differences of intensity are discussed later. Other structures, such as melanin and keratohyaline granules, are impregnanted but are not confusing except close to the epidermis.

Because this method involves prolonged treatment of the whole tissue, the bacilli are extremely well fixed and are little or not at all disturbed by subsequent embedding and sectioning. The results, it is believed, produce a wholly true picture of the distribution and appearance of the bacilli in the tissues. Ordinary methods of revealing acid-fast bacilli and methods of silvering frozen sections produce much distortion, due to shrinkages, with bacilli frequently found in positions in which they could not reasonably occur. This distortion is absent from the embedded silvered preparations. Although the nuclei of the cells are not always well demonstrated, the relation of bacilli and cells is nonetheless well shown.

### BACILLI IN THE MOST HIGHLY ACTIVE LESIONS

Such lesions are recognized histologically both from the large numbers of organisms present and from the character of the cells containing the organisms. These are not the vacuolated cells of the older leproma but are fairly simple macrophages of a wide variety of shapes and forms. In ordinary sections the parts of the cell occupied by the bacilli can be identified only by hollow areas in the cytoplasm which are not distinct vacuoles. Indeed, the numbers of organisms present would not be suspected, with the abundant and well stained cytoplasm.

In these lesions bacilli are seen in characteristic form (see Figures 1 to 4). They occur in streams, or parallel columns, and in larger cells they radiate from the central part of the cell. They look not at all like the usually described "cigar packet" or "bundle" but resemble a small colony of a fungus with hyphae streaming out from a central point. Bacilli are not matted together but are separated by well defined uniform narrow space. It becomes clear that the appearance of these fresh active lesions when stained with fuchsin methods is highly artefactual. In the latter case organisms are matted together and distorted in various ways. Hansen's original description (1874) of bacilli "crossing each other at very sharp angles" would not apply in this acute phase. This type of bacillary arrangement is limited to those lesions in which both bacilli and cells have been increas-

ing in numbers, for which reason it is of especial importance. These are the growing, young bacilli.

In these lesions the bacilli appear quite long and nearly straight. They are uniformly, but not too deeply, silvered. They show little or no beading, although occasionally there is some greater condensation of the silver at the extremities of the organisms. The manner of the division of the organisms cannot be truly determined from a study of sections, but the impression is gained that in this case bacillary cell division must take place by transverse fission.

### THE CHRONIC LEPROUS GRANULOMA

All stages between the highly and chronically active lesions are recognized. Of all lepromas that come under the microscope, the majority are of the chronic type, in which various degrees of vacuolation of the cells occur. There exists in leprology a popular misconception of the nature of these vacuoles, it being assumed frequently that they are the spaces occupied by the bacilli. This is not the case, as the silver impregnations show plainly. The vacuolated cells display a wide variation in size, the smallest with a single centrally placed nucleus, the larger with several nuclei anywhere in the cell. They are enmeshed in a reticulated, vascularized connective tissue framework, and in the silvered preparations the individual cell margin is demonstrated to a degree rarely observed in ordinary preparations. The bacilli are arranged in haphazard fashion in the cells. They lie between the vacuoles in what little cytoplasm is left in the cell, and the vacuoles are plainly seen as hollows between the meshwork of bacilli. The nucleus of the cell, which is commonly unsilvered by Jahnel's method but shown by Levaditi's, appears as a round space, with the bacilli lying closest to it markedly curved, their concave borders facing it. Bacilli in these cells show curved, bent, angulated forms of varying length, with variation in thickness of the individual bacillary body. Bacilli lying adjacent to vacuoles are also commonly curved about them.

It appears most likely that differences of appearance seen in bacilli in the chronic lepromatous lesion are partly determined by the development of the macrophage into the vacuolated cell with, at the same time, a marked diminution of the rate of growth of the bacilli. The organizing of the cells into a tissue is a factor in the shaping of the cells, which in turn affects the intracellular arrangements of the bacilli. The appearances of the chronic lesion suggest, even as do the clinical characters of leprosy, that in many cases the activity of the lesion is essentially stationary over long periods. The silvered preparations suggest that this is exactly the case, with only a slight degree of reproduction taking place in the highly vacuolated cell. The conception of the vacuolated cell as a characteristic cell of leprosy is valid, but it must be recognized as a later result of intracellular growth. Vacuoles develop as the reproductive activity of the bacilli diminishes.

### THE GLOBUS

The globus is a mass of bacilli, round or oval and sharply outlined. When large, it is often surrounded by a thinned-out giant cell, which may be so thin as to appear as a ring about the globus. The exact nature and significance of these structures and their manner of development have been much disputed, and the term is too frequently used to signify any large accumulation of bacilli in leprous tissues. From silvered preparations it is seen that the globus is indeed a characteristic structure following a definite evolutionary course.

In the fresh active lesions the structures commonly described under the heading of globi seem not to merit the term. There is no suggestion that the streams of actively growing organisms are developed into globi. In such cells the ordinary course is that in which vacuoles develop among groups of bacilli. The term "seed globi" applied to these groups of organisms by Cowdry (1) does not appear justified.

From the silver preparations, the globus appears to arise within a cell, or a group of cells fused together, after the cell has undergone degenerative changes with loss of nucleus and cytoplasm. There is always, in the bacillus-laden cell, much lipid material, which persists in the globus, as do also the vacuoles, so that the early or young globus presents much the appearance of the parent cell. Larger or older globi show fusion of the vacuoles, and the aged globus shows a single vacuole occupying most of the globus, with the bacilli distributed largely about the margin. In small young globi bacilli are routinely found well silvered. In old globi bacilli become progressively more weakly silvered. Bacilli near the rim of the globus frequently are better silvered than those more centrally placed. Sections of leprous tissues containing globi stained by fuchsin show, on the other hand, the bacilli to be equally stained throughout the globus.

The globus frequently becomes something of a foreign body. In a definite small percentage of cases of leprosy there is forma-

tion of giant cells throughout the lesions. Many of these are of the foreign body type, and they routinely enclose globi. In regressive leprous lesions the globus is seen to be a highly persistent structure, usually with some form of multinuclear cell response to it. From the silvered sections it is seen that many bacilli in globi lose the affinity for silver, suggesting an alteration of their chemical structure. This is interpreted as a degenerative change, although the bacillus is preserved morphologically.

Globi are by no means uniformly observed in leprous lesions, and circumstances leading to their formation appear to be somewhat accidental therefore. The extremely large globi rarely observed seem to arise from coalescence of many cells, not from enlargement of the formed globus. The density of organisms in the globus naturally corresponds with the density of bacilli in the cells from which the globus arose.

### DEGENERATIVE FORMS OF BACILLI

Weakly silvered and nonsilvered bacilli are readily identified in globi. Similar bacilli are also commonly encountered in minor numbers in almost every chronic lesion. They are seen in areas in which well silvered bacilli also occur, with all degrees of silvering observable. Both silvered and nonsilvered bacillary fragments are also seen. It seems clear that these are degenerate forms of the organisms. They may be fairly numerous in small areas, and their presence supports the idea that in the chronic leproma death and degeneration of bacilli are constantly taking place in some areas, even though other areas of the same lesion show evidences of progression. It is suggested that well silvered organisms are healthy organisms; poorly silvered ones, degenerate or dead organisms. Obviously, such a conclusion can be drawn only on a broad basis from those specimens in which many degrees of silvering are observed in the same section. Unless absolute uniformity of technic is maintained, there will be differences in the degree of silvering in different blocks.

Pleomorphism of the lepra bacillus is marked in the chronic lesions but barely noticeable in the fresh lesions. It is especially prominent in areas in which degenerate forms occur, and is regarded as being related to the regressive phase of leprosy, not to the growth phase of the organisms. Study of such organisms has, in the past, led to erroneous conclusions with regard to the essential morphologic aspects of the lepra bacillus.

## 17,4 León Blanco, Fite: Silvering of Lepra Bacilli 44

### HALOS SURROUNDING BACILLI

In silvered preparations a halo is routinely seen about the bacillus as a narrow clear space investing the organism. It appears not to be a phenomenon of spherical aberration. However, halos are also encountered in fuchsin-stained sections and may be observed in sections of lesions containing bacteria quite unrelated to leprosy, so that their significance seems small. It is important to stress that the halos do not fuse with the vacuoles in the cells and that they are not to be regarded as such.

## SUMMARY AND CONCLUSIONS

Lepra bacilli are readily silvered in blocks of lepromatous tissues with Jahnel's and other methods.

Bacilli silvered in the block undergo a minimum of distortion and yield a true picture of the infection of the tissues, scarcely obtainable with other methods. While such silvering is not practical as a routine clinical-pathologic procedure, it is highly valuable in the study of the lepromatous lesion.

Study of silvered sections shows that past concepts of the morphologic aspects and arrangements of bacilli are open to considerable revision. Actively growing organisms resemble minute colonies of fungi, and do not appear as "bundles" or "cigar packs." A new concept of the nature of the globus is offered.

Different degrees of silvering observed indicate that the weakly silvered bacillus constitutes one form of the degenerating organism.

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## DESCRIPTION OF PLATES

## PLATE 17.

FIGS. 1 to 4. Development of the vacuolated cells. Figs 1 and 2 illustrate the disposition of lepra bacilli in fresh active lesions. In Fig. 1 the bacilli radiate from the central part of the cell, which is occupied by the nucleus. Fig. 3, from the same section as Fig. 1 shows an older area, with beginning vacuolation and bacilli curved about the vacuoles. Fig. 4 shows a still older lesion with most of the bacilli ranged about vacuoles, or spread apart by the developing vacuoles, and moderate pleomorphism of the bacilli.



PLATE 17

## PLATE 18.

FIGS. 5 to 8. Development of the globus. Fig. 5 shows highly vacuolated cells (well filled with bacilli) sharply set apart by the organization of the lesion. There is some coalescence of vacuoles. Such cells are forerunners of globi but do not necessarily develop into them. Fig. 6 shows a well developed but young globus encased by a giant cell, bacilli well silvered throughout and moderate coalescence of vacuoles. Fig. 7 shows a still older globus with weak silvering of bacilli, and Fig. 8 shows an old globus with complete coalescence of vacuoles and faintly silvered bacilli. Note, however, that there are well silvered bacilli in the encapsulating giant cell.



[INTERNAL, J. LEPROSY, VOL. 17, NO. 4



PLATE 18