A MICROSCOPIC STUDY OF MYCOBACTERIUM LEPRAE[1]

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The microscopist accustomed to the routine examination of leprous material sees a variety of morphologically and tinctorially different bacterial forms in preparations from different patients, and sometimes a diversity of forms in a single specimen. The large number of acid-fast and non-acid-fast organisms cultivated from leprous materials, most of which differ one from another morphologically or in color production or in staining reactions, and most if not all of which fail to produce clinical leprosy in laboratory animals, makes it necessary to preface these remarks with the statement that the writer knows of no certain method of determining which of the organisms sometimes seen in leprous tissues are causal and which are commensal.

Another point of interest is that the persistence of acid-fast bacilli in the human host, who may have had clinical leprosy for many years, and the similar persistence for years of acid-fast bacilli in vitro without evidence of proliferation, causes speculation as to which of the acid-fast bacilli seen in material freshly obtained from leprous tissues are living and which, if any, are dead but resisting disintegration.

That an "acid-fast bacillus" commonly and consistently present in certain diseased tissues is responsible for the clinical entity called leprosy is almost universally accepted. There is not, however, a consensus of agreement in explanation of the variety of forms exhibited by the organism. *Mycobacterium leprae* is not infrequently referred to as monomorphic, and it is interesting to note the variety of forms which have been considered typical. Some writers, on the contrary, attempt to correlate clinical findings with the prepondence of certain bacterial forms.

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A suitably prepared specimen from leprous tissues shows, typically, acid-fast micro-organisms disposed as single rods unattached to one another, as rods within phagocytes (lepra cells), and as rods in spheroidal clumps (globi). Single organisms (Plate 1), which are usually straight with rounded ends, seldom are uniformly cylindrical but contain from one to seven metachromatic granules (beads) about twice the diameter of the unexpanded portion of the rod. Mensurational differences between rods in the same specimen are marked, but most notably in the long axis. Single rods vary in length from 1.5 to 7 microns, and rarely more; the width of the unexpanded portion of the cylinder is usually from 0.2 to 0.3 micron. In single rods having more than one bead there is an apparent relationship between the number of beads and the total length; usually the beads are about 1 micron apart.

When pairs of rods are apparently adherent, the point of attachment seems to coincide with the location of the beads. Similarly in presumed branching forms, the outshoot is attached to a bead. The mode of multiplication of the rods is not clearly evident to the microscopist. Pairs seen side by side suggest longitudinal splitting, whereas in the same specimen definitely branched or end-to-end forms may be seen.

While the rods are described as "acid-fast", there is considerable difference in the intensity of staining and resistance to decolorization. The beaded portions of the rods, when examined with high magnification, seem to have an equal affinity for the basic dyes and appear purplish rather than red when counterstained with methylene blue.

Lepra cells (Plate 2), as was recognized by Hansen, consist of both fixed and wandering tissue cells which have engulfed but have not necessarily destroyed mycobacteria. The latter continue sometimes to proliferate, in some instances growing within the intact phagocyte until it becomes moderately distended (Fig. 18). Occasionally the phagocyte is apparently killed quite soon after ingesting the acid-fast rods; and the rods, continuing to proliferate, mechanically or as the result of trauma protrude through the cell membrane (Figs. 13 to 18).

Of great interest, and of perhaps greatest diagnostic importance, are certain colonies of mycobacteria (Plate 3) which can be demonstrated readily in most cases of leprosy. Before Hansen satisfied

himself of the presence of a definite bacterial form in leprosy he saw and described certain elastic, fragile, brownish masses which were later recognized as clumps of bacilli and were designated as "globi" by Neisser. These clumps or colonies, which may measure from 10 to 100 microns or more in diameter, are composed of bacilli; these vary from a few to uncountable numbers.

In the fresh wet specimen, as in a hanging drop preparation, globi bear considerable resemblance to leukocytes in size, shape and granular appearance. In the fixed and stained preparation, however, the spheroidal masses become flattened, and if subjected to trauma during the smearing process they rupture and lose their identity. When not traumatized, globi may be seen as disc-like masses, seemingly restrained by a limiting membrane within which the outermost rods are aligned somewhat concentrically. From the periphery toward the center of the mass the organisms become more densely packed and are scarcely recognizable as individual rods.

If while the mounting medium is still fluid the microscope objective be pressed gently against the cover slip, the globular masses may be seen to rupture at one or more points. Following this occurrence single rods, unattached to one another, stream through the openings in the restraining wall. Gentle manipulation of the fine adjustment of the microscope, after some practice, will result in "pumping out" nearly all the rods, excepting the few which may have become permanently fixed to the slide (Fig. 25).

Since Hansen's original description of globi there has been considerable speculation concerning the structure and significance of these globoid masses of bacilli. Some observers have considered them to be intercellular colonies; others have considered them to be clumps formed within lymph spaces, mechanically compressed into spherical or spheroidal form; still others have expressed the opinion that the masses represent colonies of individual rods bound one to another as zooglea. A fourth view is that they may be characteristic colonies growing within an as yet unidentified restraining membrane. To this view the writer subscribes.

Passing mention is made of certain acid-fast or acid-resisting granules which are sometimes seen in quiescent or so-called "negative" cases of leprosy where bacterial forms are no longer readily demonstrable. That these granules are of significance is yet to be determined. They are considered by some as debris of mycobacteria,

and by others as representing a part of the life cycle of the *Myco. leprae.*

DESCRIPTION OF PLATE

PLATE 1.

Selected microscopic fields from the same slide, prepared from a case of leprosy, skin type, of twelve years' duration, showing considerable clinical improvement. Slide prepared by a routine method of carbol-fuchsin staining. Magnification approximately ×3500.

FIG. 1. Two club-shaped bacilli each approximately 2 microns long.

FIG. 2. Slender form, about 4 microns long, containing one metachromatic granule.

FIG. 3. One slender, multibeaded, slightly curved form, approximately 4.5 microns long, and one straight rod about 3 microns long.

FIG. 4. One slightly thicker rod, more nearly uniformly stained, approximately 2.5 microns long, with a tendency to bipolar staining; also a smaller one 2 microns long.

FIG. 5. A curved, multibeaded rod, approximately 4 microns long, and one club-shaped rod about 1.8 microns long.

FIG. 6. A curved, multibeaded rod, about 4 microns long.

FIG. 7. Three rods aligned, streptothrix-like, totalling about 10.5 microns.

FIG. 8. Four apparently different forms similar to those above shown.

FIG. 9. Pseudo-branching, three rods seemingly adherent to one another.

FIG. 10. Single rod suggestive of budding.

FIG. 11. A form believed by the writer to be branching.

FIG. 12. Similar to Fig. 11.

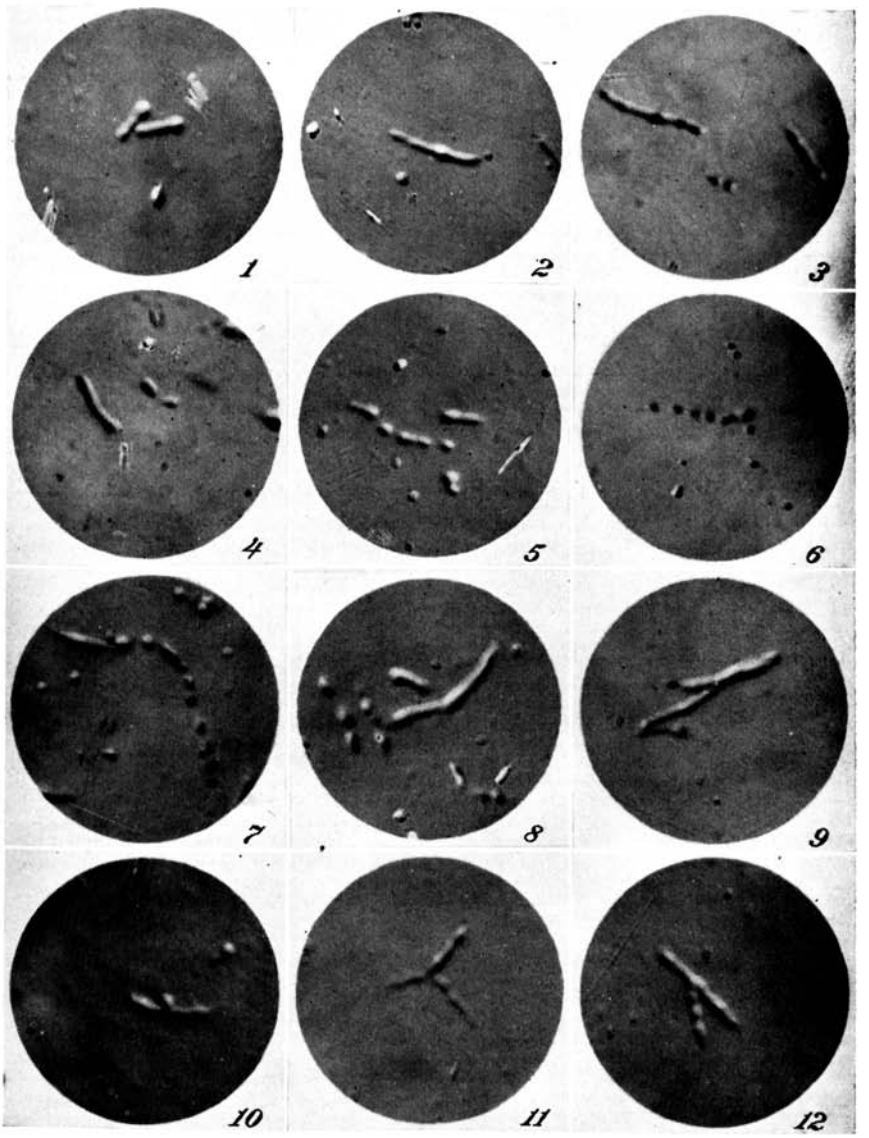

PLATE 1.

PLATE 2.

Lepra cells from selected fields in a single slide preparation obtained from an active, advanced case of leprosy of the skin type, of about ten years duration. Routine staining. Magnification about ×3500.

FIG. 13. A large mononuclear cell with inclusions of many single and clumped acid-fast bacilli. The cell wall of the leukocyte has evidently not yet ruptured and is defined by the outermost rods. The nucleus (the darker, nearly circular portion to the right) is not yet invaded by the rods.

FIG. 14. Lepra cell nearly filled with bacilli. The phagocyte is evidently dead since the cell wall has been ruptured and bacilli are protruding in all directions.

FIG. 15. Mononuclear phagocyte, evidently dead, considerably distended with bacilli which stretch and rupture the cell wall.

FIG. 16. Phagocytic cell, the cytoplasmic portion of which seems to have been entirely replaced by progressively growing rods, disposed singly and in globoid masses, compressing the nucleus into the upper left segment. The cell membrane has apparently not ruptured, and is defined by the almost concentric arrangement of the outermost rods just within the limits of the photograph.

FIG. 17. Ovoid phagocytic cell almost completely filled with single and clumped acid-fast rods. These spread over the nucleus, which occupies the lower segment of the cell.

FIG. 18. Lepra cell almost completely filled with acid-fast bacilli. Two nuclear fragments (clear spaces) remain uninvaded. The composite picture resembles, the writer believes, the "vacuolated" globus described by Hansen. An immature globus is seen above and to the left of the lepra cell.

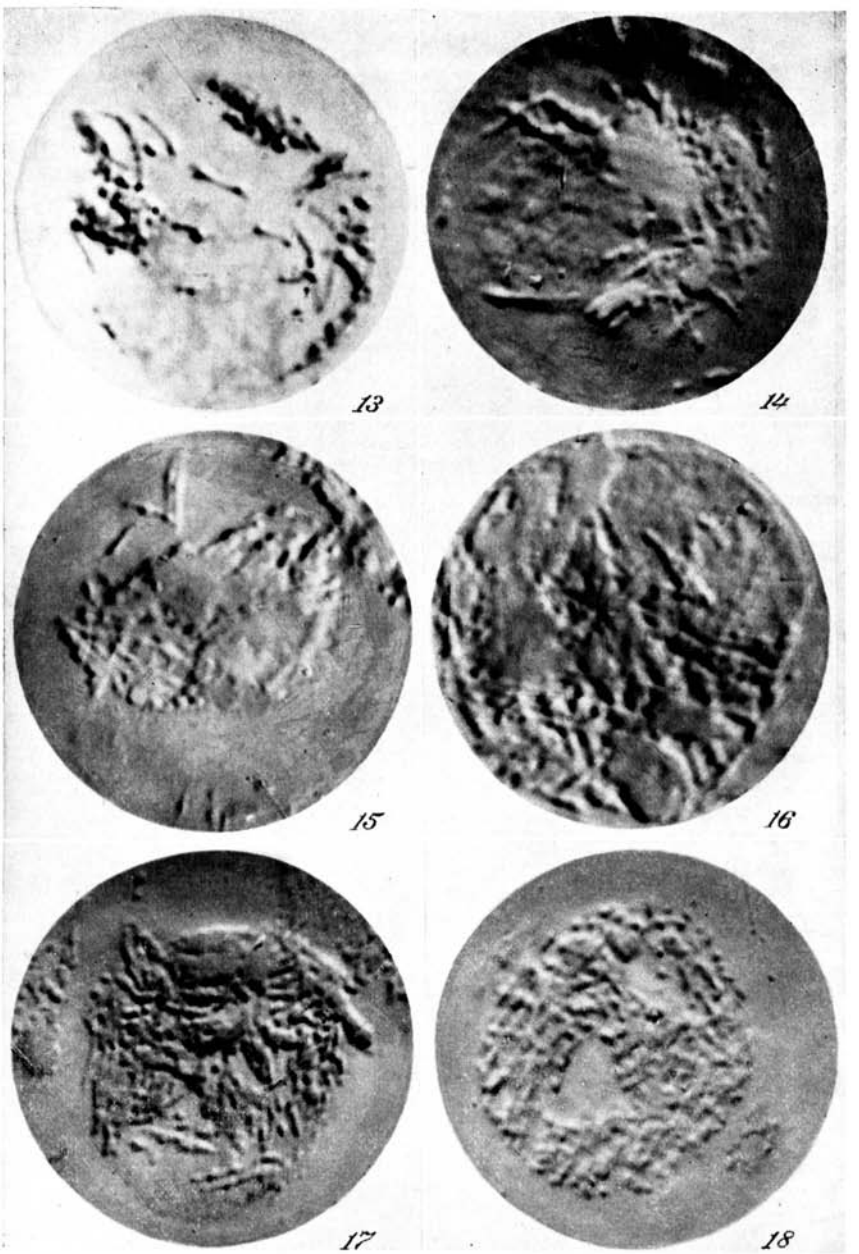

PLATE 2.

PLATE 3.

From the same microscopic slide as the illustrations in Plate 2. Magnification ×3500.

FIG. 19. An immature globus consisting, probably, of two organisms.

FIG. 20. An immature globus, somewhat distorted in fixation, consisting of perhaps three organisms. Leukocytic fragments occupy the lower left segment of the circle.

Fig. 21. A similar but larger globus, considerably distorted in fixation, consisting of approximately twenty organisms.

FIG. 22. A small globus, flattened to disc-like proportions in drying and fixation, consisting of probably several hundred organisms.

FIG. 23. A moderate sized globus almost completely filling the photographic field and consisting of uncountable acid-fast bacilli. The wall of the inclosing membrane may be surmised by the position of the outermost organisms, seemingly aligned with their long axes concentric.

FIG. 24. A similar dense globus with a pseudo pod-like projection drawn toward the right in making the preparation, evidencing the elasticity of the unfixed globus.

FIG. 25. A small globus ruptured in making the smear preparation, freed bacilli having spread toward the right half of the preparation. The original size and shape of the globus may be conjectured from the circular ring of organisms adherent in situ.

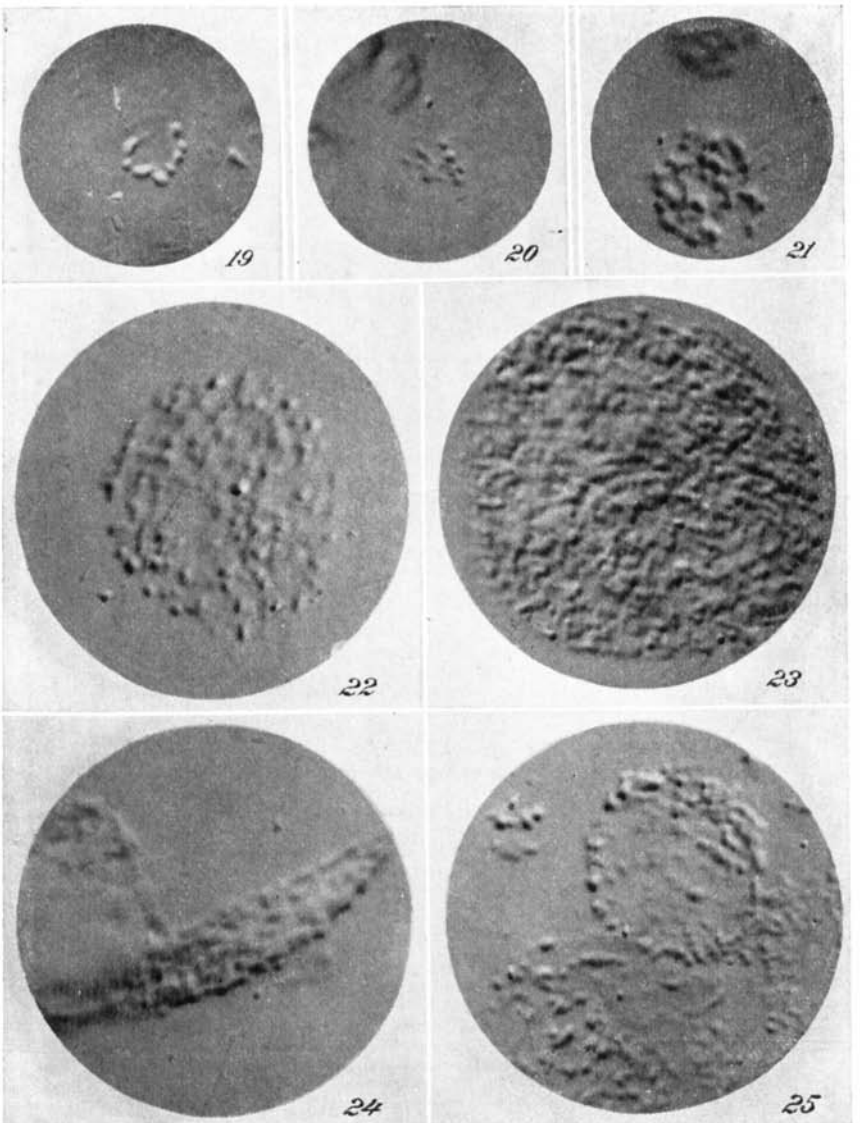

PLATE 3.