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STUDY OF THE MORPHOLOGY OF MYCOBACTERIUM LEPRAE UNDER THE ELECTRON MISCROSCOPE[1]

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The study of the morphology of *Mycobacterium leprae* by means of the electron microscope is unquestionably of great scientific interest, in view of the great difficulties of determining their structural characters with classical optics. The alterative effect of stains, and the doubts which often arise as to whether the structures which are visualized are real or are the result of reactions between the coloring agents employed and the cellular elements under study, further enhances the interest of observations of this kind. The scanty literature which exists concerning investigations of *M. leprae* with the electron microscope further justifies this first report. We have seen only one report on the subject, that of Bishop, Suhrland and Carpenter published in 1948 (1). They studied several species of the genus Mycobacterium, and presented two electron micrographs of *M. leprae* as isolated individuals, but no globi, having employed a complicated technic in their study.

The impossibility of cultivating the leprosy bacillus, and the failure of experimental inoculations in animals to provoke general alterations like those existing in human leprosy, necessitates the direct study of material obtained from leprous lesions, in such a way that the germs are caught in the morphological condition in which they exist in the only medium which permits their reproduction and development.

This first work had for its purpose the study of the morphology of the germ in patients who had had no treatment of any kind, so as to ascertain the different forms involved in its

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varied pleomorphism. It is planned later, in collaboration with Dr. E. Jonquieres, Jr., to undertake further observations with patients in the different evolutive stages of the disease and to determine the morphological changes, if any, which reveal the effects of the drugs employed upon the bacillary structure.

From classical optics, *M. leprae* is described as a bacillus whose length varies from 1 to 8 microns and its transverse diameter measures from 0.3 to 0.5 microns, the sides parallel and the ends rounded, appearing when stained in either solid or granular form. This germ, discovered by Armauer Hansen in 1871 (2), has the characteristic of lying side by side in pairs or aggregated in groups, veritable heaps, lodged either intra- or extracellularly, in the lesions it provokes in the tissues.

To observe the bacilli it is necessary to stain them by any of various techniques, based principally on their capacity to resist the action of acid and alcohol (Ehrlich, Ziehl). Believed to be more variable in size than *M. tuberculosis* (Babes), which belongs to the same genus, many attempts have been made to establish criteria for the differential diagnosis of the two. This matter does not present the same interest in electronic optics, because of the quite distinctive characteristics which the two species show.

Although the general form is rectilinear, the leprosy bacillus has also been described as slightly curved, this applying particularly to the granular forms which are ascribed to the different evolutive stages of the disease, and also to the effects of drugs upon the affection.

Rogers and Muir (3) believe that the appearance of the germ varies according to the stage of the disease, and on that basis they have established two forms. (1) One is that of the evolutive phase, in which the germs present the normal vegetative form and in which they develop without encountering organic reaction. They are solid, uniformly staining, from 1 to 8 microns in length, straight or slightly curved, arranged in parallel like cigars in a package, heaped together. (2) The other form comprises the bacilli as they occur in the reactional phase of the disease, in which—although some maintain the vegetative aspect described — many show different characters due to the irregular manner in which they take the basic stains. These forms are described as follows: (*a*) diphtheroid rods with bipolar staining; (*b*) rods containing a series of dots, giving the appearance of a string of beads; (*c*)

large, round, spore-like forms, with an attenuated rod projecting at one or both poles; (*d*) spore-like forms with no rod projecting; and, lastly, (*e*) thin, uniformly stained rods.

In this first report we are concerned only with describing the bacillary morphology which we have observed in untreated cases, which would thus correspond with the vegetative forms of Rogers and Muir. Later investigations will involve the detailed study of the forms to be found in the bacilli from treated patients, to confirm or correct the results obtained by means of the usual laboratory staining.

With respect to their group formations, we know that one of the principal characteristics of *M. leprae* consist of its arrangement like cigarette packs, or its disposition in groups which form the intra- and extracellular globi, the *gelbes* of Schollen or ring bodies of Hansen, which in some cases contain such large numbers of germs that, according to Jeanselme (4), they may even be visible to the naked eye.

There have been differences of opinion among investigators about the nature of the cement substance, called "gloea," which binds these veritable colonies together. This material is not easily made visible by common optics, it being demonstrated only by certain laboratory reactions (scarlet red, sudan III, Ciaccio), with which it behaves as a lipoid.

Finally, with respect to the differential diagnosis of the mycobacteria, particularly the distinction of the bacilli of tuberculosis and leprosy, in our opinion—as has been said—this question is not of importance with respect to electron optics because the latter germ, at least in its vegetative forms, possesses characteristics that make it easily distinguishable from any of the varieties of *M. tuberculosis,* human, avian, bovine or turtle. This has been shown in two reports on the human variety (5, 6), and in a further report shortly to be published on the avian, bovine and turtle varieties (7). The only observation of interest which can be added will be with respect to a question often raised about both *M. tuberculosis* and *M. leprae,* namely, that of the actual role of the granules found in the bacillary cytoplasm or alone. The question is whether they should be regarded as microspores. The fact that with the tubercle bacillus they have been observed in large numbers in old cultures, exhausted and unfavorable for growth and reproduction of the germs, has a parallelism with the findings with the leprosy bacillus in patients submitted to intensive and effective treatment. Lastly, we may say that in untreated pa-

tients we have been unable to demonstrate the presence of granules which closely resemble true spores.

To this résumé of the classical knowledge of the bacterial morphology of the species under study, little could be added except unimportant details, most of which have been questioned by authors of world reputation (8, 9).

METHOD AND MATERIAL

Technique.—In this work we used the E.M.U. electron microscope, at magnifications from 3,500 to 10,000 diameters.

Material studied.—This was obtained through the kindness of Dr. E. Jonquieres, Jr., from a patient in the Sanatorio Sommer de Rodriguez (history No. 1691), an Argentinian aged 38 years, with lesions of the L2 type, who had had no treatment.

In a lenticular leproma of the right forearm, a small superficial incision was made with a bistury down to the dermis, seeing to it that no capillaries were cut so as to avoid the presence of blood in the material to be examined. The lesion was squeezed with slight pressure to produce an exudate and lymph, the extraction of which was facilitated by gentle scraping. The material obtained on the blade of the bistury was diluted with distilled water to obtain a slightly opalescent suspension. Other examinations were also made with dilutions made with physiological saline, but this causes considerable interference with the investigation because of the crystallization of the sodium chloride; otherwise, the results were the same as those obtained with the suspension in distilled water.

This suspension, made by the mixture of the leprous exudate and lymph in distilled water, was placed by means of a capillary pipette on a mesh of stainless steel wire covered with a film of parlodion and placed in an incubator at 37°C. until it was dry.

The absence of any staining which would disturb the normal images, and the fact that we were dealing with living germs that had never been subjected to any laboratory technique which could affect their normal structure, increases the interest of the investigation carried out.

RESULTS

Electron microscope examination shows that the bacilli present themselves in two forms, isolated or grouped. In the latter case the numbers are variable, ranging from a few individuals to veritable colonies containing hundreds of them.

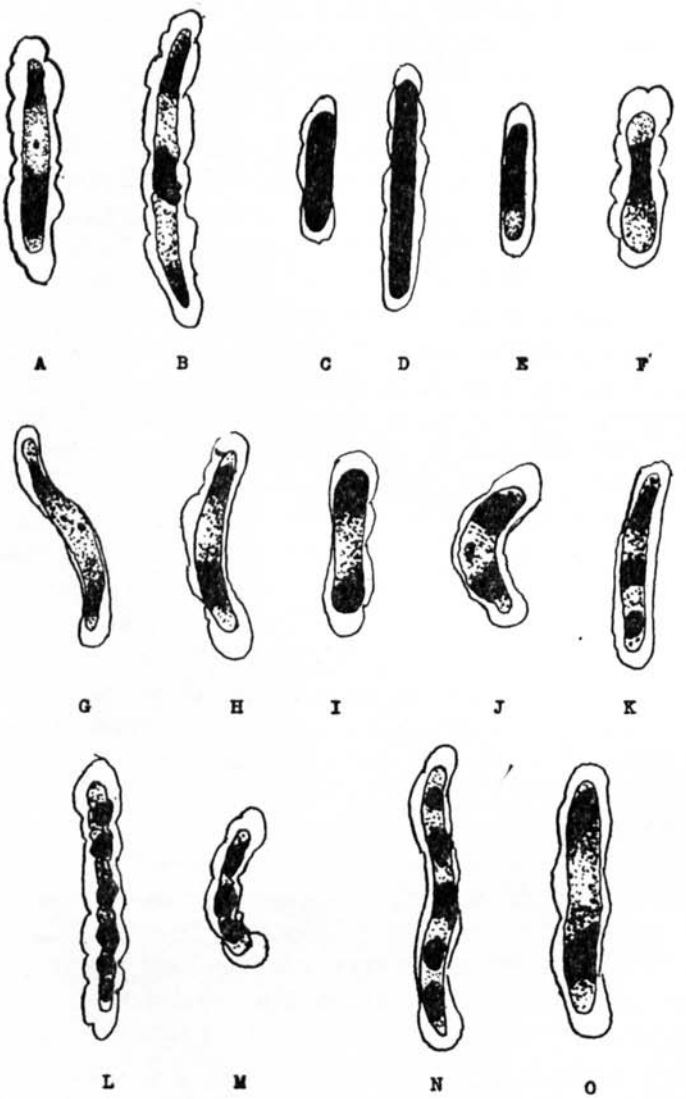
When the bacilli are isolated, they are frequently seen as forms measuring from 2 to 6 microns in length by 0.3 to 0.5 microns in width, their ends pointed or rounded and the central part somewhat thicker, with condensations of the cytoplasm toward both poles but not extending to the extreme ends and appearing rather in the nature of a rod and not of rounded granular appearance. This general conformation gives them a certain resemblance to a *"toscan"* cigar. The periphery of the bacillus shows a halo of semicircular contours which seem to isolate the germ from the surrounding material. All of these characteristics may be seen in Fig. 1.

Besides this characteristic and very common form, we found in our preparations isolated solid forms, with dense cytoplasm which is impermeable to the electron rays, but provided with a peripheral halo, and also short or long granular forms with three, four or more condensations at different points in the cytoplasm, or with a single central condensation and the ends of the bacilli showing transparent cytoplasm and a distinct membrane. We must add that in a few fields we have seen bacilli which lacked the characteristic peripheral halo.

In Fig. 2 there is shown an elongated form containing three rod-like condensations, apparently at the moment of cell division. Fig. 3 shows two isolated forms, one straight, short and dense, and the other in the form of an italic "S" with condensations in the cytoplasm. In Text-fig. 1 there are sketched some of the numerous isolated forms observed in the study.

With respect to the dimensions of the germs observed, we found that the common forms measure from 2 to 4 microns, although some which are several microns longer may be found. As for the transverse diameter, that varies from 0.2 to 0.5 microns in the narrower and the wider parts, respectively.

Although it is true that in some cases the isolated forms appear solid and dense of cytoplasm, they generally show the presence of large, rod-like cytoplasmic condensations, disposed at the poles, or near them, or in the central part, and also smaller granulations located in the thinner parts of the bacillary protoplasm. In almost all of the isolated forms we have observed the presence of a peripheral halo surrounding the cell membrane, its contours curved or semicircular, especially clear-cut at the ends of the bacilli. This halo is probably due to the gloea, since its aspect changes and it appears differently when the bacillary accumulations are studied.



TEXT-FIG. 1. Drawings showing the pleomorphism of the isolated forms of *M. leprae*. A. The very common characteristic (*"toscan" cigar*) form, with polar condensations and peripheral halo (cf Fig. 1). B. Long form, apparently beginning transverse division (cf Fig. 2). C. Short solid form. D. Long solid form. E. Solid form with a permeable segment (cf Fig. 3, left). F. Form with central condensation. G. Form of the shape of an italic S, with polar condensations (cf Fig. 3, right). H. Curved bipolar form. I. Straight bipolar form. J. Short, curved bipolar form. K. Long form with three granules in the cytoplasm. L. Multigranular elongate form. M. Short granular form. N. Long, somewhat sinuous form with numerous granulations. O. Form with condensations of the oblique type.

Regarding the investigation of the forms massed like packages of cigarettes, or heaped together, the classical globi, we have been able to make numerous interesting electron micrographs, some of which are presented here.

In Fig. 4 there is seen a group of three bacilli arranged in cigarette-pack formation, showing granulations in their cytoplasm and the peripheral halos described. Fig. 5 is of a larger number of bacilli, some of them long and in general with dense cytoplasm.

In Fig. 6 there is presented a larger group of bacilli, about 15 of them, forming an interesting skein-like mass in which can be seen long and granular forms, the whole surrounded by a peripheral halo, which gives it the appearance of a glomerulus. The fact that each of the bacillary units of large groups such as this loses its individual peripheral halo to present images which always show a total halo, leads us to suppose that it results from the fusion of the gloea produced by each bacillus, thus forming the peripheral halo which surrounds the entire bacillary mass.

Fig. 7 is of a group of bacilli in which details cannot be made out, except in those at the periphery, which are seen to have cytoplasmic condensations. In Fig. 8 there is shown an agglomeration of an even larger number of bacilli, some of which are bipolar or granular, short or long; and in Fig. 9 there is seen a large mass of bacilli in which a great number of them are in concentric arrangement, in the manner described by classical authors as seen with the ordinary microscope.

The constancy of the images which show a peripheral halo, surrounding each germ individually in the isolated forms and the grouped forms, collectively, is regarded as in favor of a true secretion, excretion, or product of bacillary metabolism which favors their agglomeration and which is identified with the classical gloea. The effect of this element in isolating the bacillus, and its relation with the as yet unsolved difficulties of its cultivation and inoculation, are interesting and important questions which the electron microscope can help greatly to clarify.

SUMMARY AND CONCLUSIONS

1. The leprosy bacilli obtained from an untreated patient with lesions of the L2 grade have been studied by means of

the electron microscope, employing magnifications ranging from 3,500 to 10,000 diameters.

2. This investigation has demonstrated that material obtained from lepromas by means of a superficial incision consists of exudate or lymph that is very rich in germs, which facilitates their observation and study.

3. This material, diluted with distilled water instead of saline to avoid trouble with salt crystals, was studied within 24 hours after it was taken, and the germs showed no evidence of degeneration.

4. The many forms observed included isolated ones and groups of varied size and appearance. Among the former, there were bacilli which were short and long, straight and curved, solid and granular, with monopolar, bipolar and multiple condensations. The grouped forms appeared as aggregations of a few individuals up to agglomerations which appeared as veritable colonies.

5. This study is to be continued to determine the characteristics of the germs in treated patients, and also in patients in different evolutive stages of the disease.

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DESCRIPTION OF PLATES

PLATE 8.

(All micrographs 3,500x before reduction.)

FIG. 1. Electron micrograph of an isolated leprosy bacillus, in material obtained directly from the patient mentioned in the text. This germ shows slightly thinned parts, with a middle zone of greater width. In the cytoplasm there are two rod-like polar condensations, and a small one in the central region. Note the presence of a peripheral halo which entirely surrounds the germ, probably arising from the bacillary secretion or excretion product, called the gloea. (Cf Text-fig. 1A.)

FIG. 2. An elongate form showing three rod-like condensations, two of them polar. The central one indicates a stage in the division of the bacillus. (Cf Text-fig. 1B.)

FIG. 3. Showing two isolated forms, one straight, with dense cytoplasm except for one thin end, with a transparent peripheral halo (cf Text-fig. 1E), the other in the form of an italic S with two larger polar condensations and smaller granules in the cytoplasm (cf Text-fig. 1G).

FIG. 4. Three bacilli in parallel (cigarette-pack formation), with condensations in the cytoplasm and surrounded by a peripheral halo.

FIG. 5. A group of bacilli, the central ones paired, which are of various dimensions, some being easily twice as long as others. Although in general their cytoplasm is dense, condensations can be seen in them. All are surrounded by peripheral halos.

FIG. 6. Showing a collection of elongated bacilli, slightly curved and granular, the whole surrounded by an over-all peripheral halo.

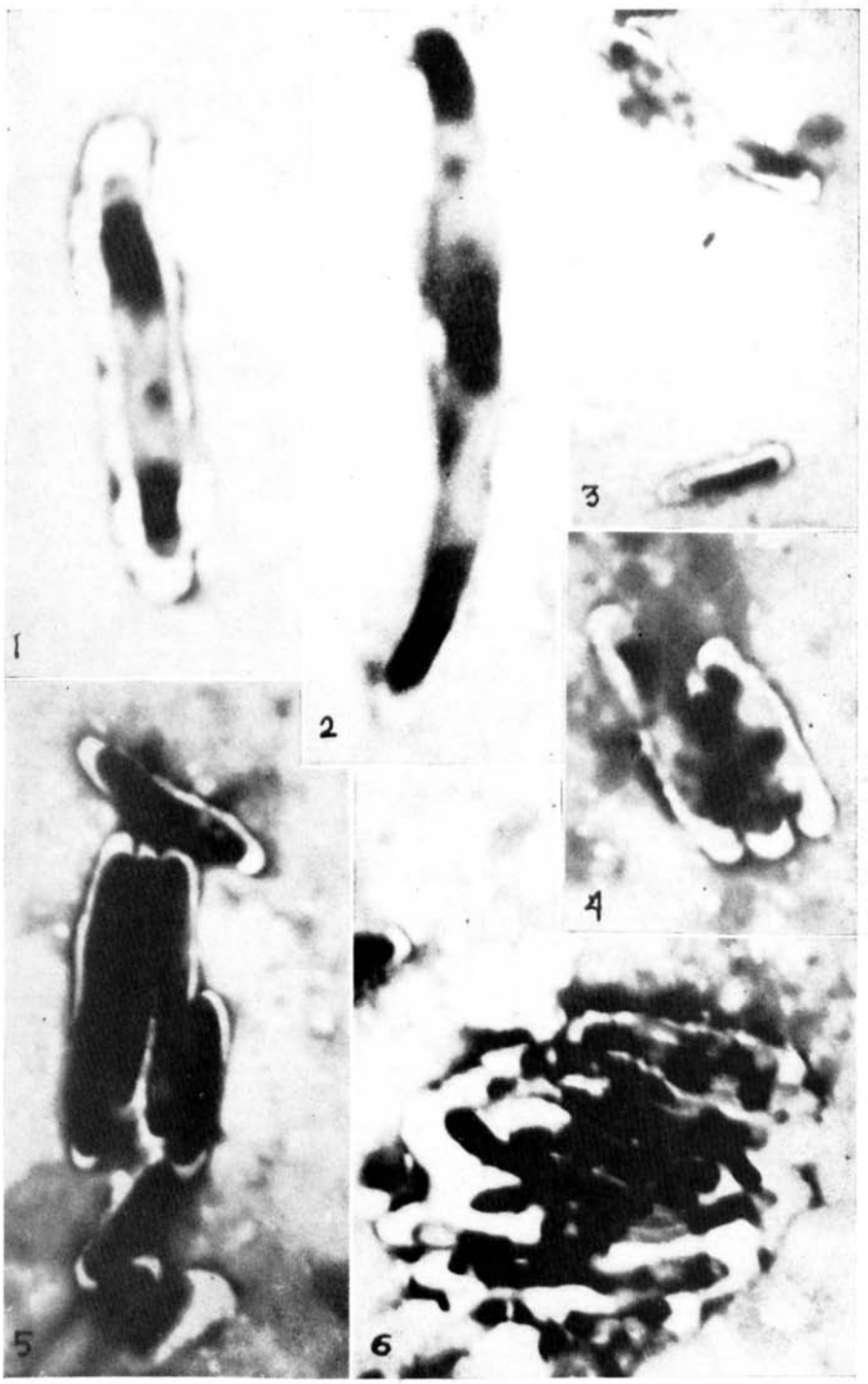

PLATE 8.

PLATE 9.

FIG. 7. A group of numerous bacilli some of which at the periphery appear granular, with the characteristic total halo.

FIG. 8. A bacillary mass surrounded by a common peripheral halo. The germs at the border show various forms and structural characteristics, whereas the central ones, because of their density and the superimposition of their images, give the impression of a bacillary ball.

FIG. 9. A bacillary globus which shows a great mass of germs surrounded by a peripheral halo. At the border several granular forms can be distinguished.

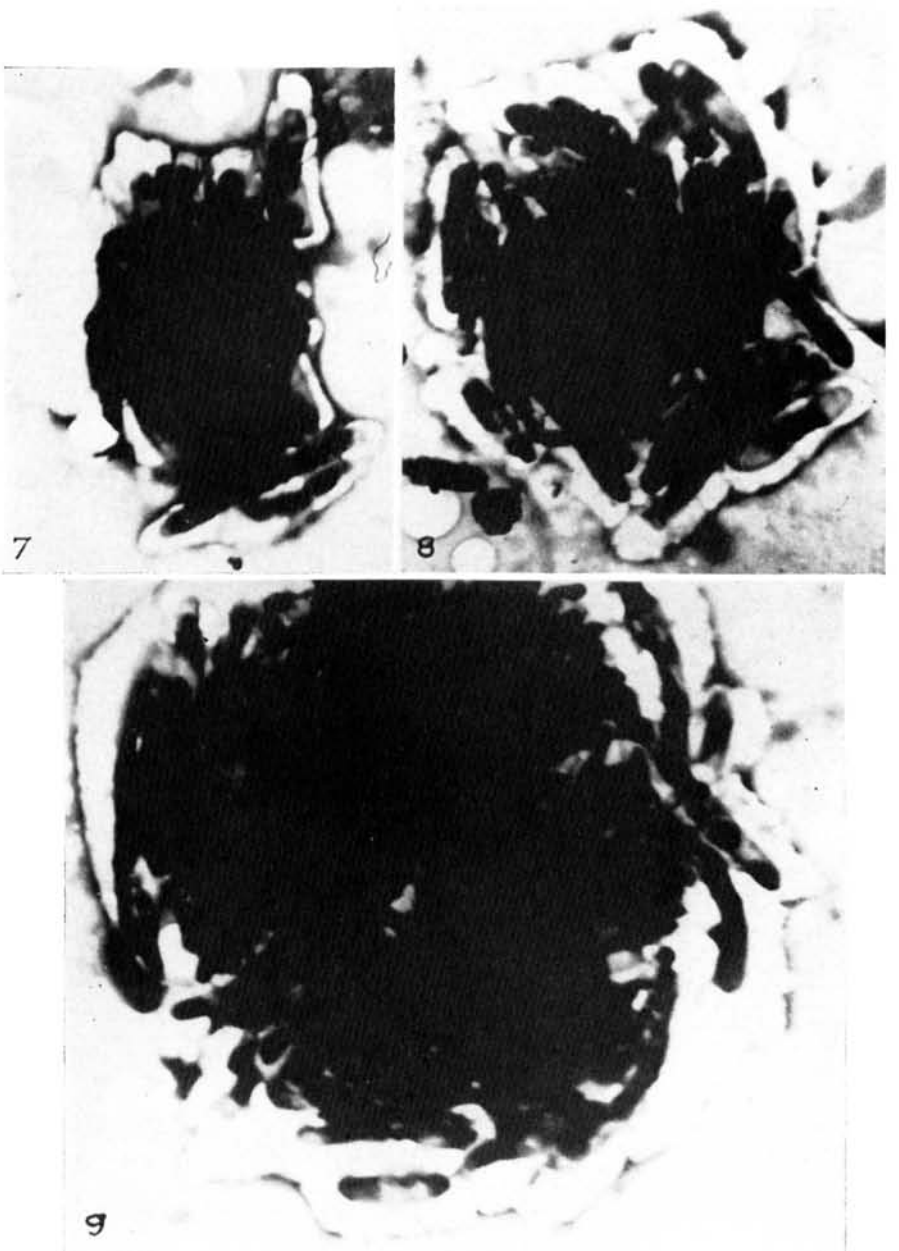

PLATE 9.