A DEMONSTRATION OF MYCOBACTERIUM LEPRAE BY ELECTRON MICROSCOPY [1]

THOMAS A. HAEDICKE

Medical Service, Walter Reed Army Hospital
Army Medical Center, Washington, D. C.

R. L. REAGAN, DOROTHY M. SCHENCK
AND A. L. BRUECKNER

Live Stock Sanitary Service Laboratory
University of Maryland, College Park, Maryland

The following is a description of the case from which the material examined by electron microscopy was taken.

A Filipino male, 15 years of age, was admitted to Walter Reed Army Hospital because of irregular shaped lesions supposedly of two months duration on the trunk and back. About one year prior to admission there had been similar lesions on the extremities, some of which had been anesthetic to pain. He had not realized the significance of these lesions and had not informed anyone of his condition. The patient was raised in the Philippines, and during the recent war lived under various conditions in the mountainous region of the islands. His only previous illnesses were the usual diseases of childhood. His mother, aged 39, and her other children are in good health. In 1947 the patient and his family were examined and given clearance for transportation to Japan. It was there that he first noticed the lesions on his extremities. Upon arrival in this country from Japan he noticed red, raised lesions on his face as well as enlarged ear lobes.

Physical examination upon admission was essentially negative except for skin lesions, which were as follows: enlarged ear lobes; reddened, raised lesions on the malar eminences; and numerous multiform, well-circumscribed, white and pink patches on the trunk and extremities. Some appeared infiltrated while others were flat, atrophic and anesthetic (Fig. 1).

The laboratory examinations of the blood and urine revealed nothing abnormal. The test for syphilis was negative. The sedimentation rate varied from 7 to 35 mm. Blood calcium and phosphate levels were normal. Stool examination showed hook worms and whip worms. An electrocardiogram and X-rays of the chest, hands, and feet were normal.

Acid-fast bacilli were found in nasal smears, and in scrapings from ear lobes and in smears from acute irregular body lesions. Biopsies of these same areas revealed acid-fast bacilli in tissue sections. The final diagnosis was Hansen's disease with lesions of both lepromatous and tuberculoid aspect.

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[1] This paper was originally scheduled to appear in Volume 19, Number 4 but had to be postponed because of the unfortunate loss of the original photographs in the process of publication.

MATERIAL AND METHOD

The technique for obtaining acid-fast bacilli from suspensions of skin scrapings for electron microscopy was as follows: A sterile scalpel was used to remove the epidermis and some of the upper layers of the skin proper from lesions on the patient's right arm (Plate 2), after swabbing with alcohol. This material was placed in a sterile petri dish and sent to the Maryland laboratory. There it was ground in a sterile mortar, using alundum for an abrasive, and diluted with 2.0 ml. of physiological saline solution. It was then spun lightly at 1000 revolutions per minute in an angle centrifuge for two minutes, and the supernatant fluid was removed and discarded. The top layer of sediment was placed in 0.2 ml. of physiological saline and this material was placed on previously prepared parlodion screens, which were left to dry until the following day. The screens were then shadowed with chromium as described by Williams and Wyckoff (2) at a tangent of ⅛, and examined with an R. C. A. EMU electron microscope. Not more than two hours elapsed between the removal of the material from the patient and the preparation of the screens.

RESULTS

The results of this examination (Figs. 1-4) are not, as regards the morphology of the microorganism, in accord with what is ordinarily seen in smears from lesions, or in successfully stained sections of biopsy specimens, or in fresh preparations examined with the phase microscope (2), under all of which circumstances they appear as rods rarely as much as 10 times as long as they are broad and usually much shorter. This is true also of the forms photographed with the electron microscope without shadowing by Bishop, Suhrland and Carpenter (1) ; the three undistorted individuals which can be measured in their pictures—as reproduced, at about 20,000x—all measure about 10 mm. broad at their widest points, but only 37, 51 and 64 mm. long, respectively.

The forms revealed in our micrographs are most extraordinary in the length of most of them, and to our knowledge they are without parallel in any pictures of mycobacteria made with the electron microscope or otherwise. Few of them appear as bacillary rods; most of them are streptothrix-like filaments, and where there are several together the effect is often bizarre in the extreme, as shown to some extent in Figs. 3 and 4.

In the upper right corner of Fig. 1 is one individual of fairly normal proportions, but not of normal morphology with respect to the darker side, there being irregularities probably ascribable to the shadowing of thicker portions of the rod. The longer one in the lower left corner is plumper and more rounded, and hence more heavily shadowed. It, too, shows variations of thickness, but its main peculiarity is the narrow, thread-like

extension of the lower end, which is not tapered. The plump solidity of this individual recalls some of those in the phase microscope photomicrographs of Richards and Wade, but none of the unshadowed forms pictured by Bishop and associates. Centrally in this picture are other relatively short forms, together with more irregular objects which would be difficult to explain unless, possibly, they represent bacterial cytoplasm without enveloping membrane.

In the upper center of Fig 2 there is another solid form of not exceptional proportions, rounded at one end but tapered at the other. Near it is a rounded body which is of interest. To suggest that it might be a bacterial granule produced by breaking off of a rounded element such as appear to the right of it, in the end of the long structure, would be pure speculation. A heavily shadowed solid form, of serpentine shape and intermediate in length between the more normal ones and the streptothrix-like strands, crosses downward from the center of the picture; it, too, is rounded at one end and tapered at the other.

Of special interest are long, filamentous structures with internal condensations. The one which crosses Fig. 2 upward from the amalgamated mass at the left is very irregular in contour, alternately thickened and narrowed, with one point where the shadowing metal accumulated densely. It has the appearance of a partially emptied membranous tube, its thinness in parts being demonstrated by the fact that the plump form which it crosses can be seen plainly through it. On the other hand the two very long forms which cross Figs. 3 and 4 are uniform in contour, the evident condensations of cytoplasm not affecting the diameter.

The morphological peculiarities demonstrated in these micrographs cannot be explained on the ground of degenerative or involutive changes occurring in the material in the short interval after the material was removed from the patient and before the screen preparations were made. Nor can the elongate strands be collagen fibers, which are of very different aspect in such preparations, or any other known formed element which might have derived from the lesion tissue. It can only be concluded that in this particular case, at the time of the examination, the leprosy bacilli had assumed most unusual forms. The observation that this microorganism can do this under any circumstances is of considerable interest.

SUMMARY

Electron micrographs are presented of leprosy bacilli from

a Filipino patient in a reactional phase with lesions diagnosed as both lepromatous and tuberculoid. The preparations having been shadowed with chromium, the appearance of the microorganisms differ from, and are complementary to, the electron micrographs of unshadowed bacilli published by Bishop and associates. In their pictures granules appear as dark bodies in lighter backgrounds; in ours they appear as prominent white masses in darker backgrounds.

The forms which we observed are for the most part extraordinary with respect to their length, they often appearing more as streptothrix-like threads than bacilli of normal proportions. They are interesting, also, in the contrast presented by different individuals with respect to their density; some appear solid and plump while others, in part or entirely within the pictures, appear as more or less empty membranes except where there are granules. This confirms in striking fashion appearances that are often suggested in stained preparations, and that have been shown by Richards and Wade in fresh preparations studied with the phase microscope, and by Bishop and associates in electron micrographs of unshadowed bacilli.

ABSTRACTO

Se obtuvo el material para exámen con el microscopio electrónico, de un paciente filepino del "Walter Reed Army Hospital," en Washington, D. C., cuyo diagnóstico clínico fué el de lepra con lesiones tanto lepromatosos (nodulares) como tuberculoides. El material se manejó estérilmente y las preparaciones se hicieron antes de dos horas después de haber sido obtenido. Después de dejarlas secar durante la noche, las preparaciones fueron plateadas con cromio.

Las micrografías electrónicas demostraron, en lugar de las formas bacilares que se ven ordinariamente, filamentos extraordinariamente largos semejantes más al estreptotrix que a los bacilos normales. La pared celular aparecía tener una estructura membranosa "vacía." Se considera interesante el que el bacilo de la lepra pueda producir éstas formas extrañas.

REFERENCES

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2. Richards, O. W. and Wade, H. W. Application of phase microscopy to the examination of the leprosy bacillus. Mem. V. Congr. Internac. Lepra, Havana, 1948; Havana 1949, pp. 517-525.
3. Williams, R. C. and Wyckoff, R. W. G. Applications of metallic shadow-casting to microscopy. J. Applied Physics **17** (1946) 23-33.

PLATE 2.

Photograph of the patient the subject of this report, showing the general aspect of the lesions. The one from which the material for examination was taken is on the right arm (scarifications). (Photograph supplied through the courtesy of Lt. Col. J. Sidney Rice, Walter Reed Army Hospital.)

PLATE 2.

PLATE 3.

FIGS. 1 to 4. Electron micrographs of *Mycobacterium leprae* from the patient shown in Plate 2. Preparation shadowed with chromium at tangent ⅛; magnification approximately 20,000x before reduction; as reduced for reproduction.

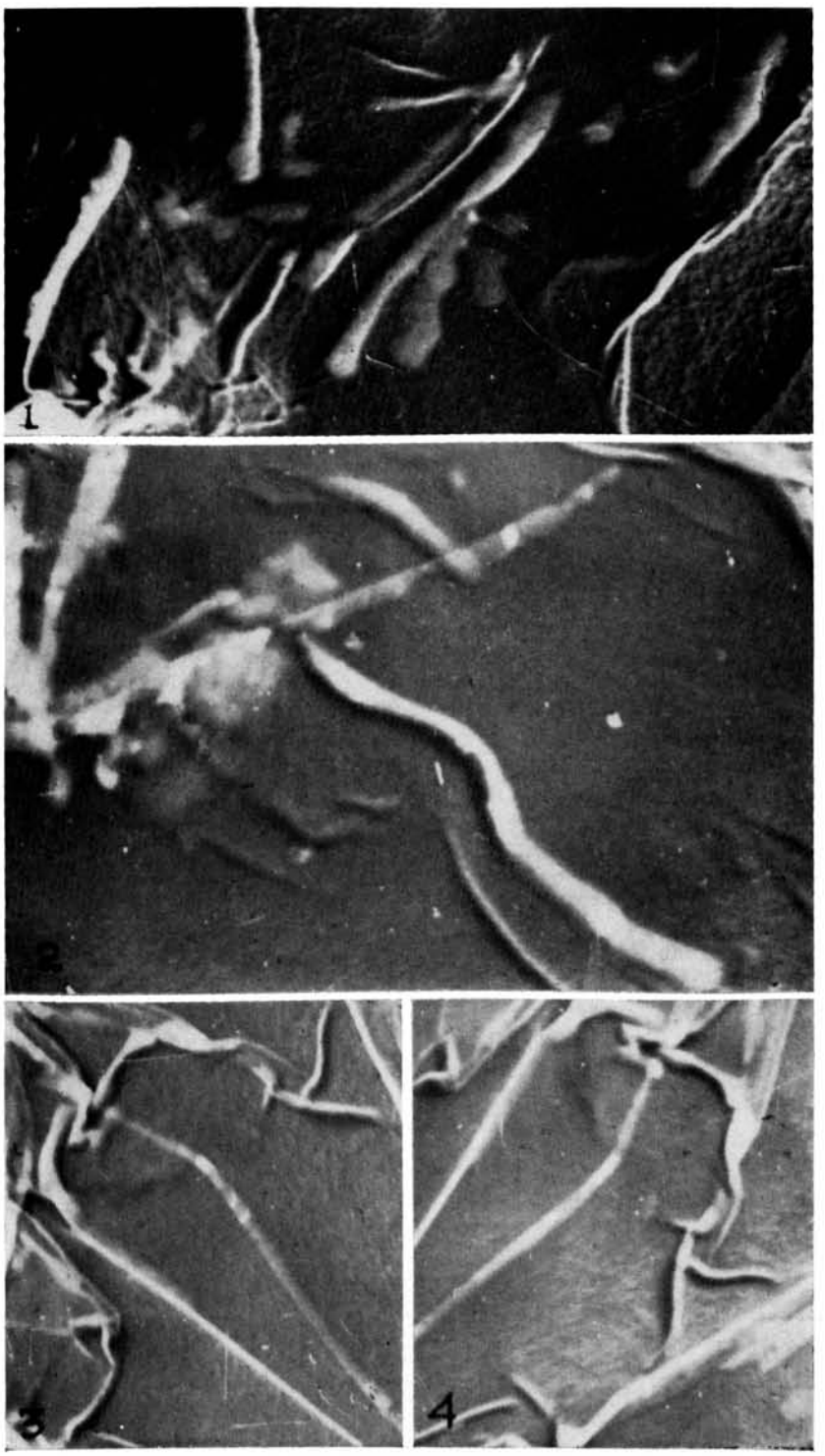

PLATE 3.