STUDY OF THE MORPHOLOGY OF MYCOBACTERIA LEPRAE BY ELECTRONMICROSCOPY*

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Studies of the morphology of leprosy bacilli have been carried out by Bishop *et al.*[1], Haedicke *et al.*[2], Malfatti and Jonquieres[3], Yamakoa[4], Brieger and Glauert[5] and by workers in India[6]. Because leprosy bacilli cannot be cultivated, the material for examination under the electronmicroscope must be obtained from lesions of leprosy patients. These lesions must be excised, minced with scissors and ground in a sterile mortar with 0.85 per cent saline. To obtain good pictures of the leprosy bacilli, this emulsion of bacilli must be made as free as possible of tissue cells, proteins, debris, etc., by repeated centrifugalisation. Lepromatous lesions rich in bacilli from patients with little or no previous treatment were first chosen for this purpose. Besides examining bacilli from lesions of untreated patients, we also examined bacilli from lesions of patients who had been treated for more than three years. Furthermore bacilli from bacteriologically postive lesions of patients with tuberculoid leprosy were examined.

It is possible, by means of the electronmicroscope, to study the internal structure of bacteria. By means of shadow casting with tungsten oxide, the surface of the bacilli can be seen.

Materials and Methods

After testing several methods, we found the following useful (mainly according to the method of Akiro Yamaoka[4] and F. W. Bishop *et al.*[1]).

A lesion, which was positive bacteriologically, was removed aseptically. It was then minced with scissors, and ground in a sterile mortar with from 3.0 to 5.0 ml. of an 0.85 per cent saline solution. After thorough grinding, it was then freed of coarser tissue particles by filtration through several layers of sterile gauze. The resulting suspension was centrifugalized for 5 minutes at a low speed. After that, 1 cc. of the supernatant fluid was taken and centrifugalized for 20 minutes at a high speed. Then the supernatant fluid was removed and discarded. To the sediment, 1 cc. saline was added and this again was centrifugalized for 20 minutes at a high speed. The supernatant fluid was removed a second time and discarded, and 1 cc. of distilled water was added to the sediment and resuspended by shaking. A loopful of this suspension in distilled water was placed on the specimen carrier of the Philips electron microscope and examined. A few specimens were shadow cast, with tungsten oxide showing the bacilli in a three dimensional way.

* Illustrations to this article are between Pages 32-33.

Discussion

The interpretation of the electronmicrographs is very difficult. The pictures are presented with the aim to collect more data which might further our knowledge of the morphology of the leprosy bacillus.

Figs. 1 and 3 show leprosy bacilli from an untreated patient. They are not homogeneous but contain irregular arrangements of dense material.

Fig. 2. Leprosy bacillus from same the patient of fig. 1, however now treated with DDS and Isonicotinic acid hydrazide for 2½ years. The bacillus seems to be swollen and transparent. Many similar bacilli were seen by electronmicroscopy of this specimen.

Fig. 4. Shows a leprosy bacillus from an untreated leprosy patient in a three dimensional way (shadow cast).

Fig. 5. The leprosy bacilli are swollen and transparent. No limiting membrane is visible. The bacilli are probably held together by a substance called " gloea." This formation might be called a globus or mass. According to Denney four different interpretations concerning the structure of the globi are given. Some observers considered them to be intercellular colonies; others have considered them clumps formed within the lymph spaces, mechanically compressed into spherical or spheroidal forms; still others have expressed the opinion that the masses represent colonies of individual rods bound together as a zooglea. A fourth view is that they may be characteristic colonies growing within an as yet unidentified membrane. Denney takes the last view. Brieger and Glauert showed pictures of groups of leprosy bacilli with and without a limiting membrane. Our picture would be more consistent with the third view. Chaussinand distinguishes between an " amas " or " mass " and a globus. In the " mass " the bacilli are not necessarily arranged parallel to one another and are still distinguishable. In the globus they should lie end to end, in parallel array and are so tightly packed that it is no longer possible to distinguish them from one another. According to Chaussinand's definition our picture shows a mass. Probably we are dealing with different phases of development of the leprosy bacillus in the human being.

Figs. 6, 7 and 10 show a leprosy bacillus from a patient with lepromatous leprosy, treated with DDS and thiosemicarbazone for 3½ years. They appear swollen and transparent. In the screen of the electron microscope similar pale swollen leprosy bacilli from those treated patients were observed. However, as can be seen in figs 8 and 9, darker types of leprosy bacilli in these treated patients occur. The leprosy bacilli of fig. 8 suggest transverse division. The leprosy bacilli of figs. 8, 9, 10 and 12 show a

peripheral halo. Malfatti and Jonquieres state that the peripheral envelope surrounding the isolated bacillary **units** and **globi,** which is always observed in preparations from untreated patients, is an index of cellular vitality and consequently of the virulence of the leprosy bacillus. Our findings show that these envelopes can still be seen in **isolated** bacilli from patients treated for more than three years. Besides in the pictures of the isolated leprosy bacilli from our untreated leprosy patients (figs. 1 and 3) there is not much evidence of a halo. The possibility that the halo is caused by shrinkage cannot be excluded. However, Indian research workers report that comparable forms were seen with the electron-microscope, the phase contrast microscope and with routine microscopy of leprosy bacilli, from the same source. Figs. 11 and 12 show leprosy bacilli in a three dimensional way from patients treated with DDS and thiosemicarbazone for 3½ years.

Figs. 13 and 14 show pictures of leprosy bacilli obtained from an untreated patient with tuberculoid leprosy in reaction. There appears to be no obvious difference from the bacilli from lepromatous patients.

Figs. 15 and 16 suggest remnants of leprosy bacilli from a patient with tuberculoid leprosy in reaction, treated for 5 months.

Summary and Conclusions

Bacteriologically positive leprosy lesions are excised, minced with scissors and ground in a sterile mortar with 0.85 per cent saline. To obtain good pictures of the leprosy bacillus, this suspension must be as free as possible of tissue cells, proteins, debris, etc. This is obtained by repeated centrifugalizing. Several micrographs were taken of shadowed and unshadowed samples from untreated and treated patients with lepromatous leprosy. Magnifications of up to 36,000 times were used. The leprosy bacilli from the untreated patients appear in most cases to possess more of the dark granular structure than do the bacilli from treated patients. The latter generally were more swollen and transparent. Micrographs were also obtained of bacilli from patients with tuberculoid leprosy. They did not differ from the bacilli of patients with lepromatous leprosy.

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