CAPSULES IN ELECTRON MICROGRAPHS OF *MYCOBACTERIUM LEPRAE*

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In studies by electron microscopy the significance of capsules on *M. leprae* seems to have been overlooked, in part for technical reasons and in part from lack of other cytologic and physiologic data as a basis for interpretation.

Three studies of washed, intact leprosy bacilli ([1, 3, 7]) failed to focus attention on the extracellular matrix. The difficulty of visualizing such structures on washed or cultivated mycobacteria is illustrated by Werner's inability to distinguish between virulent and attenuated (i.e., corded and noncorded) tubercle bacilli by searching for morphologic differences in surface components ([11]). He was able to demonstrate such structures only after washing the cells in phosphate buffer containing Tween 80. Washing the bacillary masses in hexane demonstrated that the surface components on virulent strains are distinctly different from those on less pathogenic forms. The cells of nonvirulent and attenuated strains were disorganized or ruptured. Cells of virulent strains were merely loosened or unravelled from tightly knot cords and skeins, and their electron-dense granules remained intact.

Malfatti ([8])recognized the sheath on *M. leprae* from untreated patients, probably because of drying the bacilli without washing, i.e., directly after dilution in distilled water. In his micrographs the transparent halos around many rods are delineated by an accumulation of proteins and debris outside them. A similar result is produced when India ink, nigrosin or Congo red is employed as a surface coat for light microscopy ([5]). De Souza-Araujo ([10]) also emphasized that halos appear around *M. leprae* from untreated patients. Brieger and Glauert ([2]) refer to intact rods from untreated patients as being imbedded in an amorphous matrix. Their Figures 3 and 4 suggest that about one-half the mass of microcolonies is comprised of an electron-transparent material which tends to keep the rods separated.

In a further study on the effects of sulfone therapy, Malfatti and Jonquieres ([9]) noted the loss of capsules, and suggested that this defect and decreased pathogenicity might be interrelated.

Ultra-thin sections of *M. leprae* show two features which have been demonstrated by assay of dye permeability and by light microscopy after surface coating. These are: (*a*) failures to preserve internal

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structures ([2]) due to impermeability of the superior bacilli to fixatives ([6]), and (*b*) loss of capsules during sulfone therapy. In Brieger and Glauert's study ([2]) of bacilli from a patient who relapsed after treatment, their Figures 5 and 6 show capsular outlines on three bacilli; Figure 7 shows that the bacilli in clumps did not lie in close contact. Brieger's Figures 9 and 10 in Cochrane's recent text ([4]) illustrate the differences in spacing and in extracellular matrix on bacilli from an untreated and treated patient, respectively.

The capsular structures on *M. leprae* from untreated patients can be seen even more clearly in the images published by Yamamoto, Nishiura *et al.* ([12]). Their Figure 5 shows capsules as transparent zones around clusters of bacilli from a patient in whom treatment had failed, while Figure 8 shows similar structures around individual bacilli from an untreated patient. It will be noted again that the bacilli do not lie contiguously, but are separated by a matrix. Their Figures 3, 4 and 7 show bacilli from treated patients. If compared with Figures 5 and 8, the absence of electron-transparent halos and the close approximation of the rods make a striking contrast.

The more statistical observations of McFadzean and Valentine ([7]) on formalinized, intact bacilli show, instead of capsules, the uniform opacity of "normal" *M. leprae;* also that the proportions of extracted, "degenerate" organisms rise during sulfone therapy. This increase of degenerate forms coincides with the high permeability to dyes ([5]) and is substantiated by the irregularity of granule distribution and structure as revealed after incubating such bacilli in crystal violet for 1 hour,

This brief survey indicates that the electron-transparent capsules on *M. leprae* have, for the most part, been demonstrated by negative outlining (proteins, debris and sectioned cell substance), just as with the surface coats employed for light microscopy; also that similar conclusions are reached by the two methods. For general purposes, assay of dye permeability and examination of surface-coated preparations by light microscopy offer certain advantages: the simplicity of preparation and of studying the effect of experimental variables on bacilli *in vitro;* the availability of dyes possessing different properties; the feasibility of comparing bacilli from a series of patients on single slides or coverslips; the ready enumeration of sufficient bacilli to describe a bacterial population more completely.

It will be noted that a relationship between impedence of growth and the loss of capsules from *M. leprae* has been illustrated in electron micrographs with astonishing regularity. In view of the small proportion of electron micrographs which can be published, and the fact that nonencapsulated bacilli occur in untreated patients, one wonders whether the presence and absence of capsules may have been an unrecognized basis for the selection of micrographs. A further review of

micrographs in the files of investigators might disclose clinical states in which similar losses of capsules occur without treatment.

SUMMARY AND CONCLUSIONS

This brief survey reveals that the extracellular capsules and matrices on *M. leprae* have been an interesting, although usually overlooked, feature in electron micrographs of this organism. Since these structures are electron transparent, they have not been recognized on intact bacilli which were washed, but have been delineated in cruder preparations where dried proteins and tissue debris provide the negative-outlining effects of the opaque surface-coats employed for light microscopy. In ultra-thin sections, the "electron-transparent zones" between clumped rods and between the surface of bacilli and the sectioned tissue cell substance have not been recognized to be the capsular components of the bacilli. The need for more adequate attention to these capsular structures has been emphasized by making specific reference to micrographs which demonstrate the consistent loss of capsules following sulfone therapy. Further studies are needed in order: (*a*) to learn whether capsular structures are impaired also during spontaneous recoveries from disease and (*b*) to elucidate the probable associations between capsules and the pathogenic potential of the bacilli. Correlations between the results of electron microscopy and those obtainable by the simpler methods of light microscopy have been emphasized.

Addendum. In studies by phase and electron microscopy, N. Mukherjee (Ann. Rep. of the Calcutta School of Tropical Medicine, (1957-58), 122-126) observed a gloea-like substance around bacilli and especially globi from untreated patients. This material disappeared after six months of sulfone therapy.

RESUMEN

Revela este breve estudio que las cápsulas y matrices extracelulares observadas sobre el *M. leprae* han constituído una característica interesante, aunque por lo general inadvertida, de las microelectronografías de este microbio. Como estos elementos son electronotransparentes, no han sido reconocidos en los bacilos intactos que habían sido lavados, sino que han sido delineados en preparaciones más toscas en las que las proteínas desecadas y los desechos histológicos suministran los efectos demarcadores negativos de las capas superficiales opacas que se emplean para la fotomicroscopía. En cortes ultradelgados, no se ha reconocido que las "zonas electronotransparentes" entre los bastoncillos aglutinados y entre la superficie de los bacilos y la sustancia histocítia en cortes son los componentes capsulares de los bacilos. Se ha recalcado la necesidad de prestar atención más adecuada a estos elementos capsulares haciendo mención específica de las micrografías que demuestran la constante pérdida de cápsulas a continuación de la sulfonoterapia. Se necesitan estudios ulteriores a fin de: (a) averiguar si los elementos capsulares se lesionan también durante las curaciones espontáneas de la enfermedad; y (b) dilucidar las probables relaciones entre las cápsulas y la patogenicidad potencial de los bacilos. Se han recalcado las correlaciones entre los resultados de la electonomicroscopía y los obtenibles con los métodos más sencillos de la fotomicroscopía.

Addendum. En estudios con la microscopía en fases y electrónica, N. Mukherjee (Ann. Rep. of the Calcutta School of Tropical Medicine, (1957-58), 122-126) observó una sustancia parecida a glea alreador de los bacilos y en particular de los globos procedentes de enfermos no tratados. Esta materia desapareció al cabo de seis meses de sulfonoterapia.

REFERENCES

1. Bishop, F. W. and Suhrland, L. G. A comparative study by electron microscopy of the morphology of *Mycobacterium leprae* and cultivable species of mycobacteria. Internat. J. Leprosy **16** (1948) 361-364.
2. Brieger, E. M. and Glauert, A. M. Electron microscopy of the leprosy bacillus: a study of submicroscopical structure. Tubercle (London) **27** (1956) 195-206.
3. Chatterjee, K. R., Das Gupta, N. N. and De, M. L. Observations on the morphology of *Mycobacterium leprae* by ordinary optics, phase microscopy, and electron microscopy. Internat. J. Leprosy **23** (1955) 385-391.
4. Cochrane, R. G., Editor. Leprosy in Theory and Practice. Bristol, England: John Wright & Sons, Ltd.; Baltimore. Md.: Williams & Wilkins; 1955.
5. Hanks, J. H. Significance of capsular components of *Mycobacterium leprae* and other mycobacteria. Internat. J. Leprosy **29** (1961) 78-87.
6. Hanks, J. H. The problem of preserving structures in pathogenic mycobacteria by conventional methods of fixation. Internat. J. Leprosy (in press).
7. McFadzean, J. A. and Valentine, R. C. The examination and the determination of the viability of *M. leprae* by electron microscopy. Leprosy Rev. **23** (1960) 6-11.
8. Malfatti, M. G. Study of the morphology of *Mycobacterium leprae* under the electron microscope. Internat. J. Leprosy **20** (1952) 95-104.
9. Malfatti, M. G. and Jonquieres, E. D. L. Study of the morphological modifications of *Mycobacterium leprae* during chemotherapy. Internat. J. Leprosy **21** (1953) 323-330.
10. de Souza-araujo, H. C. The morphology of *Mycobacterium leprae*. Leprosy Rev. (1959) 80-84.
11. Werner, G. H. Electron microscopic studies on the cellular morphology of tubercle bacilli. Adv. Tuberc. Res. **4** (1951) 53-90.
12. Yamamoto, T., Nishiura, M., Harada, N. and Imaeda, T. Electron microscopy of ultra-thin sections of lepra cells and *Mycobacterium leprae*. Internat. J. Leprosy **26** (1958) 1-8.