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EDITORIALS

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UNDISCUSSED PROBLEMS OF THE BACTERIOLOGY OF TUBERCULOID AND BORDERLINE LEPROSY

There is no overt problem with respect to the bacteriology of lepromatous leprosy, apart from the question of how so many bacilli, for the greater part more or less degenerate or dead, can be harbored in the lesion cells without causing more tissue damage than they do or exciting tissue reactivity. The lesions are supposed to be positive for bacilli from the start—i.e., when the early indeterminate macule decides to become lepromatous—and to remain positive until long after visible infiltrations have subsided. Relapse is explained on the ground of the observed long persistence of bacilli in out-of-the-way places, especially the nerves.

The situation is quite otherwise in tuberculoid leprosy. The torpid lesions of active but nonreactional cases, whether of minor or major degree, are typically negative for bacilli on smear examination, yet they slowly advance. It is assumed that bacilli are actually present, but so few that they are difficult to demonstrate. John Lowe, who discussed the etiology of the tuberculoid lesion at some length, stated that by use of his special method of staining[1] and by examining multiple sections when necessary, he had demonstrated bacilli in large proportions (60 or 70%) of lesions.[2,3] He was satisfied, as other leprologists seem generally to have been, that all of the manifestations are ascribable to the presence of the ordinary acid-fast organism in association with marked reactivity of the tissues, and not to any filter-passing or other form of the organism. There is, however, no indication in his publica-

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[1]LOWE, J. A note on the staining of *Mycobacterium leprae* in tissue sections. Indian J. Med. Res. **22** (1934) 313-315.

[2]LOWE, J. A study of macules of nerve leprosy with particular reference to the 'tuberculoid' macule. Leprosy in India **8** (1936) 97-112; Leprosy Rev. **4** (1937) 69-85.

[3]LOWE, J. A note on tuberculoid changes in leprosy as seen in India. Internat. J. Leprosy **4** (1936) 195-199.

tions that he had dealt with other than reactional lesions, the kind which leads so many of the patients at the Calcutta clinic to present themselves. There has not, to my knowledge, been any special study of this matter confined strictly to torpidly progressive lesions.

Bacilli are usually to be found in smears from reactional lesions of tuberculoid leprosy, although—at first, at least—in numbers too small to account for the extent of the tissue response even if the influence of allergic reactivity is postulated. It is in the early stages of relapse reactions in tuberculoid cases that the situation becomes a high-grade mystery most difficult to explain, although it has not been particularly discussed in any publication of which we are aware.

Let us take, as an example, the relatively uncomplicated case[4] of a young girl who, a decade before she was seen at the Manila clinic (i.e., while in her early "teens"), had had on the left cheek a solitary tuberculoid patch, evidently of major grade, which had subsided spontaneously. Since the patient did not present herself on that occasion, it is not known whether or not that lesion was bacteriologically positive, or how long it took to subside; the period of latency is therefore unknown, but presumably it was long.

Suddenly, and for no known reason, there appeared on the left side of the face a large, acute reactional lesion ("relapse reaction") which did not affect the "immune area" representing the original lesion but was abruptly thickest immediately around it, and also with a few metastatic lesions elsewhere. Even a month later, when the patient was seen, all smears were negative for acid-fast bacilli. (The fact that in most of its circumference the main lesion thinned out and merged with the normal skin in the manner of a borderline lesion, instead of being clear-cut and equally thick at the outer edge as reactional tuberculoid lesions regularly are, is not pertinent to the present discussion.)

The questions that arise are these: Where and in what form had the etiologic agent lurked during the period of quiescence? In what form did it exist after activation, when—a month or so after its appearance—no acid-fast bacilli could be demonstrated in any of the lesions present? Why was the face lesion especially thick and prominent immediately around the immune area, as it typically is there and elsewhere in the outwardly well-demarked "mother lesion" of reactional tuberculoid leprosy? These are questions which, so far as we are aware, have not been raised before, and which cannot be answered on the basis of present knowledge.

The condition suggests that the causative agent, whatever its form, may have persisted in the immune area and, on activation, spread explosively into the surrounding skin tissue, especially concentrated

[4]WADE, H. W. The first phase of borderline transformation. The so-called "relapsed tuberculoid" condition. Internat. J. Leprosy **28** (1960) 105-112.

immediately around that area—and also was disseminated by the blood stream, to produce the metastatic lesions which, although few in the particular case cited, appear as a shower of lesions which often are numerous and sometimes extremely so.[5]

It is, however, difficult to imagine the causative agent persisting in the form of bacilli in the original lesion area, which had become thoroughly immune to reactivation. It is quite as difficult to imagine the agent, in whatever form, coming from some other location to produce such local effects.

The lesion suggests that the skin around the central area might have been peculiarly sensitized to the etiologic agent, while that area itself had become immune. Dr. Marion B. Sulzberger,[6] when consulted, replied in effect:

"I agree with the suggestion that the tissues just around the primary lesion may have been particularly hypersensitized, whereas the actual site of the healed primary lesion was immune, and that the disseminated lesions appeared when mycobacteria which had been lurking somewhere arrived in hypersensitive tissues.

"This, of course, is hypothetical until one finds in the bloodstream the disseminating microorganisms or their products, and demonstrates the existence of tissue hypersensitivity and tissue immunity in the appropriate places to fit the hypothesis."

It is to be said that when a questionnaire was sent out asking for information as to the results of comparative testing in leprosy cases, with lepromin or any other antigen, of *(a)* immune areas, *(b)* immediately neighboring skin, and *(c)* normal skin at a distance, there was so little response that it seems doubtful that such tests have ever been made.

To return to the question of the form of the causative organism in the latent phase after the subsidence of the primary lesion, which in most spontaneously-healed cases in the young means permanent healing. It seems unlikely that it could have persisted in an "L" form, or protoplast, not reduced in size but without protecting cell wall and not recognizable in smears or sections, for such a naked form would presumably be vulnerable. Nor could it have been in an acid-fast granular form of microscopic size, for anything of that kind would long since have been observed. There remains, then, the question of whether or not there exists a submicroscopic form of the leprosy bacil-

[5]See, for examples, Figs. 31 and 32 of the second of Lowe's reports cited, of a case with "hundreds" of lesions on the body, face and extremities (and what may have been the "mother lesion" on the right upper arm); and the striking pictures of a patient seen by Kinnear Brown in Uganda and shown in the article by Wade referred to.

[6] Author of the course of lectures entitled *Dermatologic Allergy* (published in Springfield, Illinois, by Charles C Thomas, in 1940), who in 1927 discovered in the blood stream the fungus elements which produced the metastatic "id" lesions at the time of an allergic inflammatory reaction in the original lesion [Arch. Dermatol. & Syphilol. **18** (1928) 891-901].

lus—presumably filterable. That possibility has been discussed from time to time in the past (e.g., Lowe[2]), as it has been with respect to the tubercle bacillus, but in view of the facts that the leprosy bacillus is not cultivable and that the production of lesions in animals has been entirely unsuccessful until recently, speculation on the matter has long since been discontinued.

We had entertained the hope that by electron microscopy of ultrathin sections of tuberculoid lesions, as done especially by Nishiura, of Kyoto, and more recently by Imaeda, also from Kyoto but now working in Caracas, some such form—if it exists—might be demonstrated. Neither of those investigators has recognized anything of the sort, although on a couple of Nishiura's pictures sent us there are queries written in against certain very small unidentified bodies in the cytoplasm of the lesion cells.

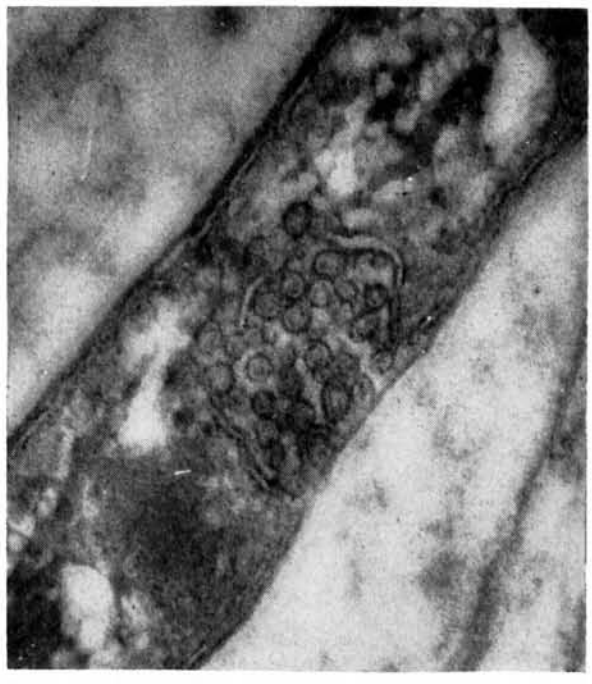
Here, however, we wish to call attention to certain suggestive features of some of Imaeda's recent pictures. Employing an improved technique of preparation, that worker has obtained remarkable pictures of intact bacilli in borderline lesions, which material has been found to be particularly favorable for the purpose, as reported in two articles the second of which is in this issue.[7,8] As first reported elsewhere,[9] Imaeda's pictures show, immediately inside a well-differentiated bacillus cell wall, a double-layered "plasma membrane" which in many instances invaginates into the bacterial cytoplasm. There it forms "intracytoplasmic membrane systems," which may be simple or very intricate (see Fig. 5 on page 408 in this issue). These are supposed to constitute one of the bacterial organelles related to metabolism, and also to be concerned with multiplication—the latter being the point of present interest. In the process of fission of the bacillus (see Fig. 10 on page 411 in this issue) there first appears in its cytoplasm a transverse cleft, the opposing surfaces of which are lined by the plasma membrane but not by the cell wall itself—at least, apparently, not until separation occurs.

One extraordinary micrograph of Imaeda's is of a longitudinal section of a single bacillus at 200,000× magnification, that body nearly 13-1/2 inches long. That picture is to be used by him in another publication, but we take the liberty of producing here a portion of it on a reduced scale (about 50,000×; see cut). On one side the plasma membrane is invaginated to form a relatively large, roughly rectangular pocket, or sack, the depth fully two-thirds the width of the bacillus

[7]Convit, J., Lapenta, P., Ilukevich, A. and Imaeda, T. Experimental inoculation of human leprosy in laboratory animals. I. Clinical, bacteriologic, and histopathologic study. Internat. J. Leprosy **30** (1962) 239-253.

[8]Imaeda, T., Convit, J., Ilukevich, A. and Lapenta, P. Experimental inoculation of human leprosy in laboratory animals. II Electron microscope study. Internat. J. Leprosy **30** (1962) 395-413.

[9]Imaeda, T. and Convit, J. Electron microscope study of *Mycobacterium leprae* and its environment in a vesicular leprous lesion. J. Bact. **83** (1962) 43-52.



body. The wall of this pocket is composed of the entire plasma membrane, and the gap above the invagination is covered only by the cell-wall layer, which appears to be intact and distinctly striated vertically.

The special feature of the picture is that the pocket appears to be filled with round to ovoid bodies, averaging about 6 mm. in diameter at the 200,000× magnification, which apparently have arisen from a layer of the plasma membrane—the inner one with respect to the pocket, but originally the outer one in contact with the cell wall. These particles, which might be taken for virus bodies, number 27 in the level of this particular section (not counting those obscured by overlapping). Each is surrounded by an outer layer, which seems to be of about the same density as, but less width than, the corresponding layer of the plasma membrane. Inside that capsular layer in each of these bodies is a narrow paler zone, and within that zone a mass suggestive of a cytoplasmic substance fills the rest of the body. The wall of the pocket is ruptured at two points in its deeper part, and several of the tiny bodies appear to be escaping or to have escaped into the adjacent bacterial cytoplasm.

This specimen is unique in definition and particularity, but similar rounded structures appear in association with the intracytoplasmic membrane systems in other pictures of bacilli made by Imaeda, and the queried structures in the microorganisms of Nishiura referred to resemble them closely. The temptation is strong to regard them as ultramicroscopic forms (''sporulets'') of the bacillus, of the order

of what we had hoped might be found in such lesion material to help explain the mysteries of the reactional lesions here discussed. They might, possibly, also explain why Convit and associates succeeded in producing lesions in hamsters with that material but not with lesions from lepromatous cases, in which the bacilli are mostly degenerated.

The possibility that these bodies might represent a vegetative form was suggested to Imaeda, but he would have none of it. He explains these apparently individual bodies as cross sections of a group of tubular elements of the intracytoplasmic membrane system. It is admittedly presumptuous of us to regard that explanation as inadequate, but we do. The original picture has been sent to the Armed Forces Institute of Pathology in Washington for an opinion, which requires consultation with experts and has not yet been forthcoming.

It can be expected that the findings of the investigators in Venezuela will stimulate others who have access to suitable clinical material, and the necessary equipment, to further such studies. It remains for the future to tell—if it will—just what the true nature and significance of the peculiar bodies described may be.

Apart from all that, however, we may repeat that it is at least evident that the borderline condition deserves more recognition and attention than it has received heretofore.—H. W. Wade