Elimination of the Acid Fastness but not the Gram Positivity of Leprosy Bacilli After Extraction with Pyridine

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Received for publication 18 February 1971

Bacilli from a number of tissues excised from cases of lepromatous and borderline leprosy, unlike mycobacteria, were found to lose their acid-fastness after treatment with pyridine.

The bacilli which Hansen saw with difficulty in osmium-fixed preparations from leprosy patients (4, 5) were found by Neisser (employing specimens provided by Hansen) to be easily observed in tissues stained with Gentian Violet or fuchsin (7). Neisser assumed that, since the bacilli from several patients had the same appearance, they must all be the same. Once it was discovered that leprosy bacilli, stained with carbol fuchsin, were resistant to decolorization with acidic ethanol (i.e., acid-fast), the common identity of leprosy bacilli seemed established. Therefore, in the minds of the men concerned, all leprosy bacilli were considered the same and, for purposes of discussion, were termed "The Leprosy Bacillus" (9). The Leprosy Bacillus was assumed to be a mycobacterium (4, 9) and penultimately came to be designated as the species form, "Mycobacterium leprae" (10; Bergey's Manual of Determinative Bacteriology, 7th ed.).

Recently, Campo-Aasen and Convit (2) described a modification of Baker's Acid Hematein Test (3) as a cytochemical method for distinguishing between "M. leprae" and M. lepraemurium. The distinction was made on the basis of a pyridine-extractable, acid hematein-positive material which was associated with "M. leprae" but not with M. lepraemurium. By the procedure of Campo-Aasen and Convit, we had no difficulty in demonstrating the presence of a pyridine-extractable, acid hematein-fixing material associated with harvested leprosy bacilli, similar to what they had found associated with bacilli in tissues from leprosy patients. Pyridine-extracted bacilli, however, were found to lose not only their capacity to fix acid hematein but, more striking, their acid-fastness. In addition, it was found that extraction with chloroform removed the capacity to fix acid hematein but did not remove acid-fastness. Subsequent extraction of leprosy bacilli and strains of valid species of mycobacteria with a number of solvents revealed that, although the acid-fastness of leprosy bacilli was removable, that of true mycobacteria was not. The present communication describes the use of a pyridine extraction procedure in the examination of leprosy bacilli from cases of leprosy.

Fresh tissues biopsied from lepromatous and borderline cases of leprosy were provided through the generous cooperation of Jacinto Convit (Chief, Division de Dermatologia Sanitaria, Caracas, Venezuela) and Leandro V. Uyguanco (Director, Bureau of Disease Control, Manila, Philippines, by arrangement with Chapman Binford, Medical Director, The Leonard Wood Memorial, Baltimore, Md.). Dounce homogenizers were used for initial grinding of biopsied samples. After homogenization, the larger tissue fragments were removed by sedimentation, either after standing or after low-speed centrifugation. Bacilli were then concentrated by differential centrifugation. Dried smears of bacilli, prepared on clean glass slides, were fixed in Bouin's fluid and extracted with pyridine according to the modification of Baker's method described by Campo-Aasen and Convit (2). Extracted and nonextracted (control) smears were stained by the Ziehl-Neelsen (hot) carbol fuchsin method and decolorized for 1.5 min with 3% (v/v) HCl in 70% (v/v) ethanol. No counterstain was used. Acid-fast bacilli were present in 19 smears from 21 cases of leprosy. Of the 19 acid-fast positive samples, 15 lost their acid-fastness after extraction with pyridine. All 15, however, remained gram-positive. One of the smears containing bacilli, whose acid-fastness was unaffected by pyridine extraction, had been pre-
pared from tissue from which we cultivated an acid-fast bacillus. The acid-fastness of this bacillus along with that of *M. tuberculosis* H37Rv, *M. intracellularle* ATCC 13950, and tubercle bacilli in sputum from an active case of tuberculosis, was apparently unaffected by pyridine extraction. Such extraction appeared to have little effect on the integrity of the walls of either leprosy bacilli or true mycobacteria, since each, after pyridine treatment, remained gram-positive. It is clear, therefore, that the basis for the acid-fastness of most leprosy bacilli and that of mycobacteria is quite different.

Four years ago, it was reported for the first time that leprosy bacilli harvested from lepromatous tissue possessed a phenolase activity not found in mycobacteria (8). More recently, a similar phenolase activity was reported to occur in bacilli cultivated from cases of both lepromatous and tuberculoid leprosy (1). The results presented here indicate that the acid-fastness of most leprosy bacilli, unlike that of true mycobacteria, can be attributed to a fuchsin-retaining material easily removed with pyridine. This material might well originate from the interaction of the host with the bacilli. If the deposition or evolution of such fuchsinophilic, acid-fast material does result from the interaction of the patient and any of certain kinds of bacteria, one might expect to find around the world a diversity of organisms capable of taking on such acid-fastness and thus becoming leprosy bacilli. When the bacilli present in leprosy patients are mycobacteria, they would be doubly acid-fast, having, in addition to the acid-fastness of true mycobacteria, that pyridine-soluble acid-fastness described here.

Currently, we are concerned with the nature of the fuchsinophilic, acid-fast material discussed in this paper and its relation to the bacterial cell and its possible role in the pathogenesis of human leprosy.

This investigation was supported by contract U-1682 from the Health Research Council of New York.

**LITERATURE CITED**


