bovine serum albumin (D-BSA) can be used as the antigen to isolate and purify this antibody on an affinity column. Inclusion of such a standard would enable the results to be expressed in mass units (mg/ml).

Similarly, the results of the two other specific, quantitative diagnostic tests ([2, 3]) can also be expressed in mass units by using known quantities of the respective monoclonal antibodies. Since each of these methods looks at one epitope, the use of such standards is logical since both the test and the standard would follow the same kinetics in the assay.

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Can *Mycobacterium leprae* Enter the Body Through Unbroken Epithelium?*

To the Editor:

Current concepts of the transmission of leprosy emphasize the importance of the nasorespiratory tract as a route of entry of *Mycobacterium leprae*. However others, for example Leiker, believed that leprosy is more likely transmitted via the skin ([3]).

In one of our earlier studies in nude mice, *M. leprae* entered the nasal mucosa through unbroken epithelium ([1]). An experiment was conducted to find out whether *M. leprae* can penetrate unbroken skin.

A fresh suspension of *M. leprae* containing 1.17×10^9 organisms per ml in Hanks' balanced salt solution was prepared. Twelve 6-week-old nude mice were used for the study. Much of the keratin layer was removed from the skin of the dorsal aspect of the right hind foot in all of the 12 mice using 6 to 8 strokes of Scotch tape. The animals were then anesthetized and both hind foot pads were anchored onto a board, taking care to expose all the dorsum of the hind feet for the experiment. Ten microliters containing 1.17×10^7 *M. leprae* were dropped over the skin of the dorsum of both feet so as to cover an area of approximately 5 mm in diameter. The suspension was allowed to dry under a gentle flow of warm air for 20 to 30 min. Once it dried, a drop of 6% gelatin in water was dropped onto the site and allowed to dry for 15 min. The nude mice were sacrificed at 8, 24, 48, 72, 96, and 120 hr after exposure to *M. leprae*, and a 4-mm punch biopsy of the dorsum of both feet was carried out. The tissues were fixed in 10% buffered Formalin for 48 hr and were processed for paraffin sections. Ten serial sections of 5-μm thick were made from each specimen, and these were stained with a modified Fite's stain ([2]). Every field in all of the sections was examined under a light microscope using an oil immersion lens ($\times$1000).

In the right feet of all animals where an attempt was made to remove the keratin layer, one or more layers of keratin still remained in all of the sections. At 8 hr, there

* Wayne M. Meyers, M.D., Ph.D., kindly served as Editor in regard to the submission, review, revision, and acceptance of this manuscript.

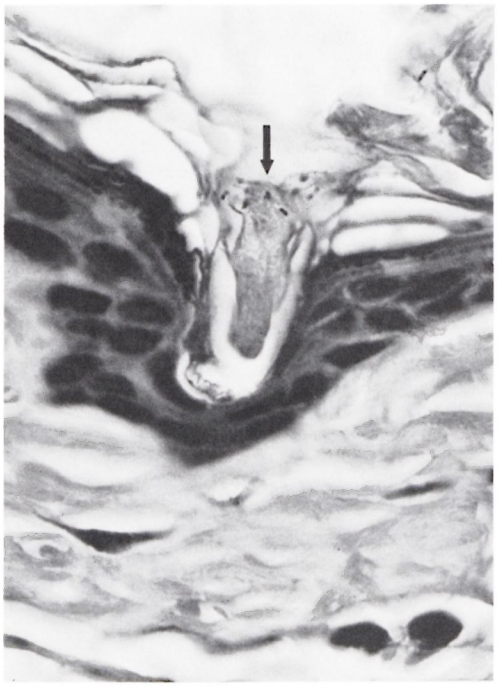

FIG. 1. *M. leprae* in keratin at 24 hr (left foot) [Acid-fast stain (AFS) ×930].

FIG. 3. *M. leprae* inside epithelium of outer root sheath of hair follicle at 48 hr (left foot) (AFS ×930).

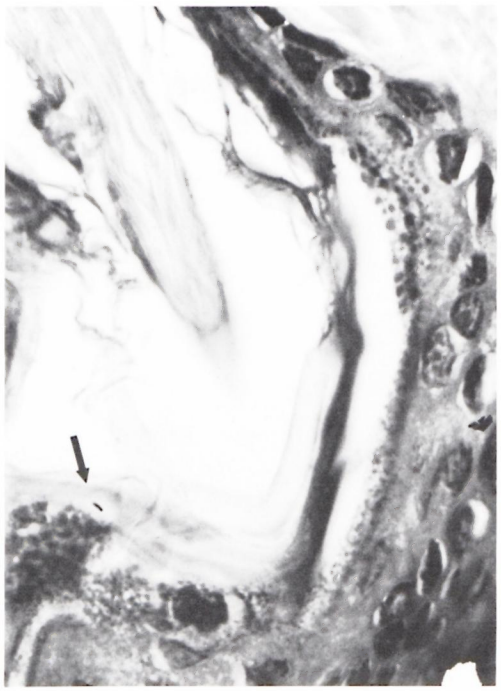

FIG. 2. *M. leprae* inside keratinized inner root sheath of hair follicle at 48 hr (left foot) (AFS ×930).

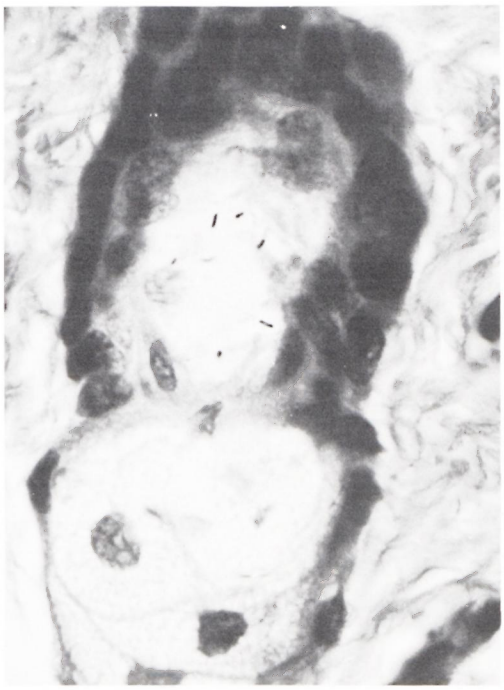

FIG. 4. A small collection of *M. leprae* inside hair follicle at 72 hr (left foot) (AFS ×930).

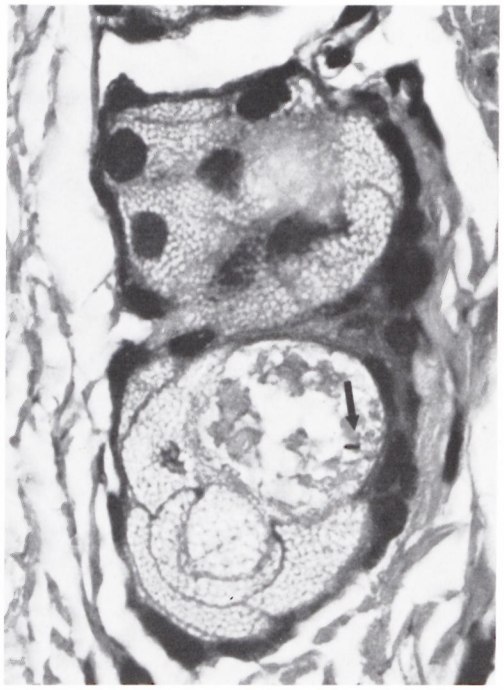

FIG. 5. *M. leprae* inside a sebaceous gland at 72 hr (left foot) (AFS ×930).

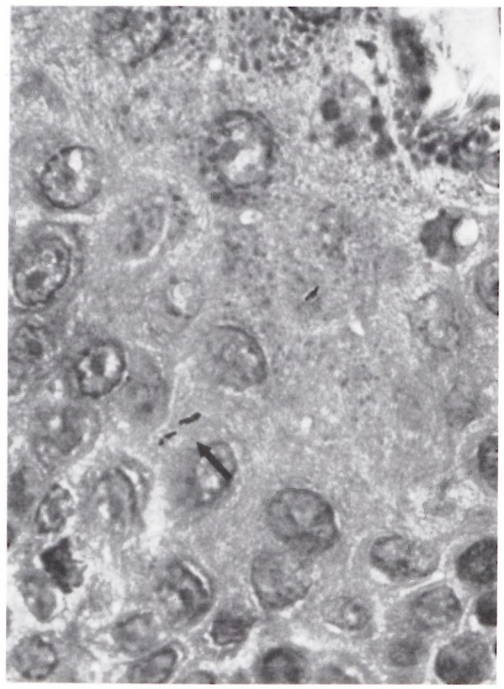

FIG. 6. Several *M. leprae* inside an epithelial cell of epidermis at 120 hr (left foot) (AFS ×930).

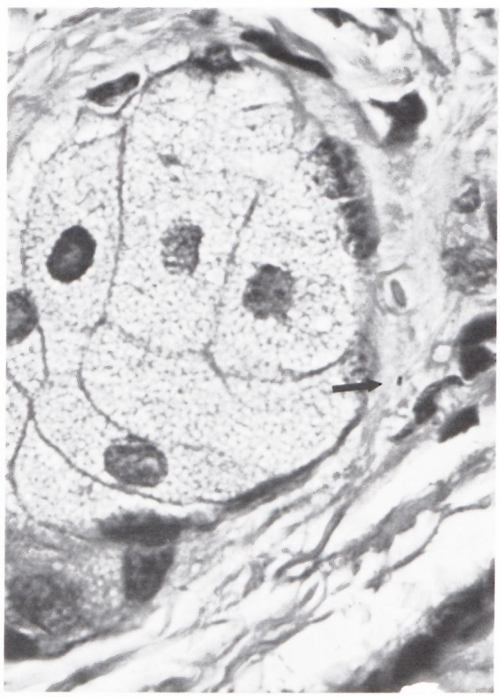

FIG. 7. *M. leprae* in connective tissue around a sebaceous gland at 120 hr (right foot) (AFS ×930).

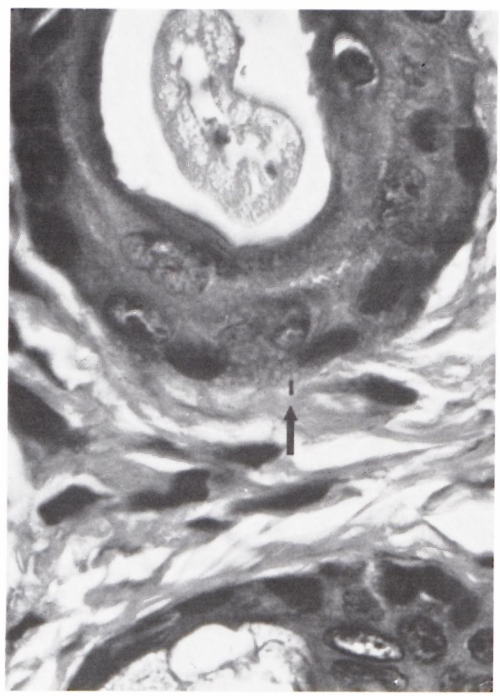

FIG. 8. *M. leprae* in connective tissue of hair follicle at 120 hr (right foot) (AFS ×930).

was a mild acute inflammatory reaction showing small scattered collections of neutrophils in focal areas in the subepithelial tissue. The keratin layers showed many acid-fast bacilli (AFB) entangled in them.

At 24 hr, the AFB continued to be seen in the keratin layer, especially in the crypts of hair follicles (Fig. 1). The neutrophilic infiltration was less but was present in some of the sections.

At 48 hr, AFB were detected in the keratin layer, especially in the region of hair follicles, and in the keratinized inner sheath of the hair follicle (Fig. 2). In a few sections bacilli were seen in the epithelial cells of the outer root sheath of the hair follicle (Fig. 3). In one specimen a bacillus was seen near the basement membrane of the epidermis.

At 72 hr, the neutrophilic infiltrate was no longer seen. The AFB persisted in the keratin layer and inside the hair follicles (Fig. 4). Organisms were also seen inside sebaceous glands (Fig. 5). At 96 hr, a few bacilli were seen in the keratin layer inside the epithelial cells of the outer root sheath of hair follicles. There was no evidence of inflammation. At 120 hr, a few acid-fast organisms were detected in the keratin layer, epithelial cells of the epidermis which was in continuation with the epithelial cells of the outer root sheath of the hair follicle (Fig. 6), in the fibrous connective tissue surrounding sebaceous glands (Fig. 7), and in the hair follicle (Fig. 8).

There was no significant difference in the histopathological appearance of skin of the right foot in which much of the keratin was removed and that of the left foot with intact skin. Although the tissues were processed in clean solutions which had not been previously used, the possibility of the organisms being displaced from the keratin surface to underlying tissues by the microtome knife cannot be ruled out.

In this light-microscopic study, *M. leprae* were seen in the keratin layer, inside epithelial cells of the skin, and the outer root sheath of many hair follicles. AFB were present even in the fibrous connective tissue surrounding the sebaceous gland and in the hair follicle 120 hr after the skin surface was smeared with 10^7 *M. leprae*. From these findings, we believe that *M. leprae* can enter the epidermis of the unbroken skin, especially through the hair follicles and sebaceous glands. The keratin layer seems to offer a barrier to the entry of the bacilli.

Many of the AFB detected in the skin were fragmented and granular. This would imply that dead organisms are also taken into the skin and that entry is a passive process. If this happens in a normal animal, it can be sensitized, desensitized, or infected depending on the dosage, frequency of entry, and viability of the organism. Electron-microscopic studies are underway to confirm these findings.

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