not show any organisms in organs such as the lungs, liver, spleen or ears.

One possible reason for the lack of multiplication may be that the skin at the flanks is warmer than that at the foot pads. It is reasonable, therefore, to suggest that although *M. leprae* may enter into the body through any skin site it can multiply and disseminate only in a suitable environment with a temperature less than normal core body temperature. The other reason may be that unlike the foot pad, the region of the flanks is a large area and the injected *M. leprae* may have fanned out without being localized and are thus lost to further evaluation.

The absence of granuloma or any histopathological changes at the right flanks of the animal examined at intervals of 12, 15, and 21 months, although AFB were harvested from the left side, is difficult to explain. The fact that even minimal inflammatory changes were absent suggests that the biopsy might have missed the site completely.

Further experiments are being done to confirm this study since the number of mice used here was small, and there were no control animals infected in the foot pads using the same inoculum and the same dosage for comparison.

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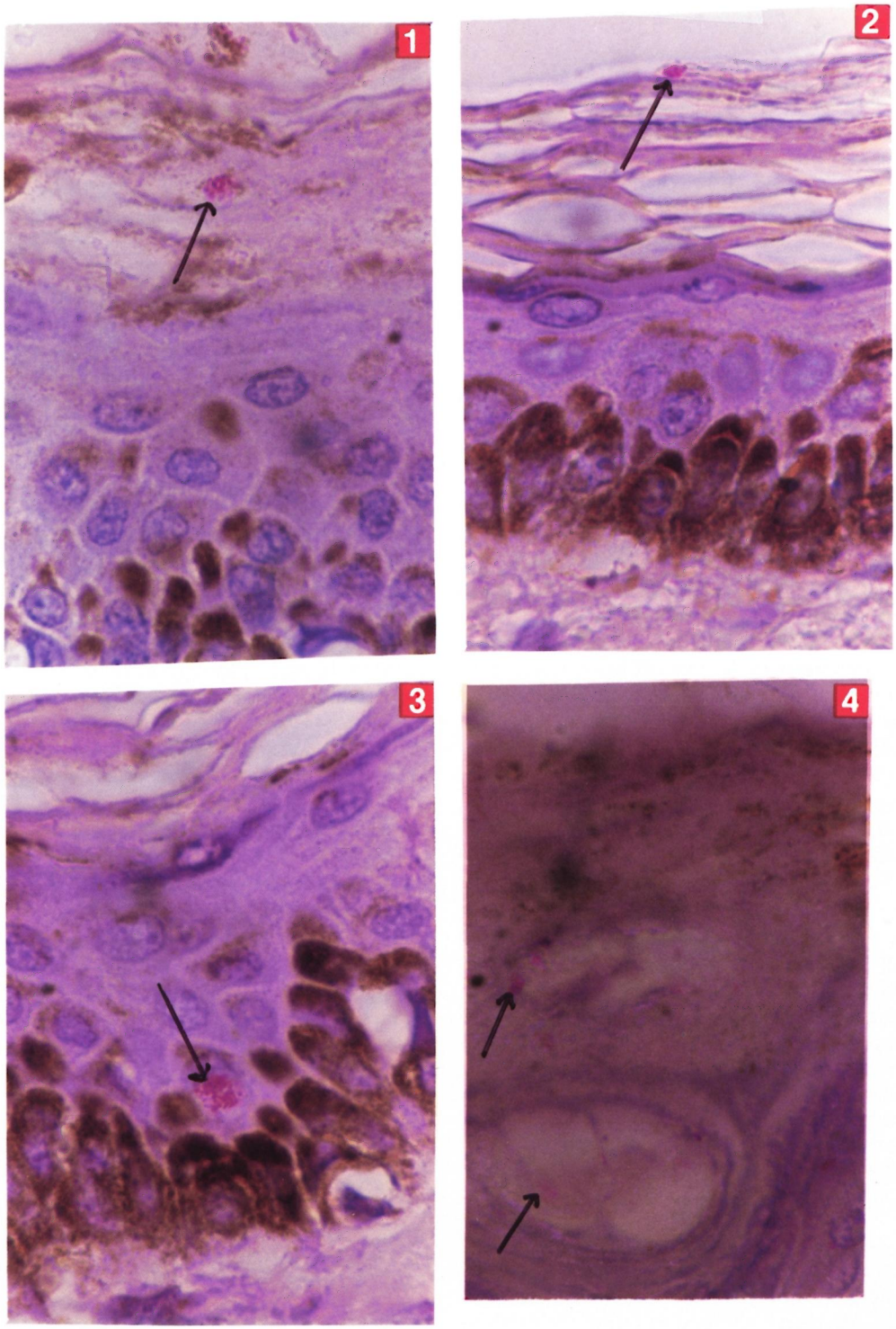
"Large Numbers" of *Mycobacterium leprae* are Discharged from the Intact Skin of Lepromatous Patients; a Preliminary Report

To the Editor:

In 1970 Pedley in an innovative study of the skin of leprosy patients concluded that the number of *Mycobacterium leprae* discharged from the intact skin of lepromatous patients was negligible and, therefore, nasal secretions were the major source of infection ([6]). Although his findings seem to be widely accepted and quoted, no attempt has been made to confirm or disprove his hypothesis. In the method employed in his study, called composite skin contact smears (CSCS), a glass slide was pressed 10 times repeatedly on the skin lesions of lepromatous patients with a high bacterial index (BI), heat fixed, stained for *M. leprae,* and examined under a microscope with an oil immersion lens. From 11 patients, 34 CSCS were studied and only 20 acid-fast organisms (AFB) were detected. On the basis of this study he concluded "that very few bacilli, if any, are discharged from the intact skin of a lepromatous patient."

In this study under report, biopsies from 13 patients belonging to the lepromatous



FIGS. 1–4. Photomicrographs showing clumps of AFB (→) in the keratin layer (Figs. 1 and 2), in the epithelial layer (Fig. 3), and at the exit of a hair follicle (Fig. 4) (H&E ×800).

group with a BI of 4+ and above were randomly chosen from the biopsy files of St. Thomas Hospital and Leprosy Centre, Chettupattu, India, from 1992 to 1998. Fresh sections were cut from the stored paraffin blocks and were stained for AFB using a modified Fite's method ([4]). AFB were looked for under an oil immersion lens in the epithelial and keratin layers of the epidermis. Whenever AFB were found, their locations were carefully studied. In one newly diagnosed, untreated lepromatous patient, a modified CSCS method was employed to obtain smears. A glass slide was coated with a thin film of water-soluble glue in an area covering 2 square centimeters. The glue-coated area was pressed against the selected skin lesion 10 times, heat fixed, stained for AFB, and examined under an oil immersion lens. Modified CSCS were obtained from eight sites.

Of the 13 biopsies studied, in 5 there were clumps of AFB in one or more focal areas of the keratin layer of the epidermis numbering over 10 per oil immersion field (Figs. 1 and 2), in 4 there were less than 10 per field, and in 4 no bacilli were detected. In four biopsies there were intracellular collections of AFB in the cells of the basal layer and of the squamous cell layer (Fig. 3). On careful study it was found that AFB were mostly found around the pores in the skin, especially at the exit of the hair follicles (Fig. 4). there were also many bacilli in the cells of the hair follicles and around the hair shaft. In the one untreated lepromatous patient in whom the skin was studied using the modified CSCS technique, large numbers of AFB were found as detailed below:

Right earlobe	Neg.	Right forehead	13
Left earlobe	85	Left forehead	39
Right upper arm	24	Left loin	35
Left upper arm	79	Left thorax	Neg.
	Total	275	

Since the glue used interfered with the staining of AFB, such studies were not done in more patients. Further investigations are being planned to refine the method of detecting AFB in the superficial part of the skin.

In this study it is clearly shown that AFB were present in fairly large numbers in the keratin layer of the epidermis of 9 out of 13 lepromatous patients. AFB were also found in the epithelial cells of the epidermis. In one newly diagnosed lepromatous patient numerous AFB were demonstrated on the surface of the skin using a modified CSCS technique. In 26 to 42 days epidermal cells can travel from the basal layer to the horny layer and, in due course, they are keratinized ([3]). Obviously, AFB would come up to the keratin layer travelling inside epithelial cells and would be shed into the environment along with keratinized cells. It was also observed that the presence of AFB was concentrated around the pores of the skin, especially those of the hair follicles. It is apparent that the organisms would exit along with the sebaceous secretions. It seems that the method used earlier by Pedley ([6]) (pressing a glass slide against the skin) was not successful in taking up the bacilli which were present in large numbers in the superficial keratin layer of the skin.

In 1964 the majority of leprologists believed that the portal of entry of *M. leprae* is through the skin, although some believed that the organism entered through the upper respiratory tract ([1]). It was also thought that direct skin-to-skin contact is likely to be more effective for transmission of the disease than indirect contact ([5]). Transmission through the respiratory route gained importance following Pedley's conclusion that hardly any *M. leprae* were discharged into the environment through the skin ([6]) and the demonstration of enormous numbers of AFB from nasal secretions of lepromatous patients ([2]).

In conclusion, this study shows that *M. leprae* are found in the superficial keratin layer of the skin of lepromatous leprosy patients and are shed into the environment in large numbers, thus challenging Pedley's findings. Direct skin-to-skin contact with such patients is more likely to transfer viable *M. leprae* from a patient to a contact than by any other method. An extensive study is being planned and its results will be reported in due course.

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Staging Nerve Involvement in *M. leprae* Infection

TO THE EDITOR:

It is assumed that leprosy is primarily an infectious disease of the peripheral nerve even though skin manifestations remain an important clinical sign of the disease. The nerve may be affected alone (pure neural) or may be the first site to be involved (primarily neural), the other possibility being concomitant skin and nerve involvement.

Briefly, *Mycobacterium leprae* infection cannot be conceived without nerve involvement. Leprosy neuropathy is essentially a neuritis. The inflammatory reaction within the nerve contributes to the pathogenesis of leprous neuropathy through a variety of effector mechanisms ([8]). It is assumed that in leprosy an immunopathological mechanism plays an important role in the pathogenesis of nerve damage ([5]). Staging this nerve involvement is important in order to find a new therapeutic approach for the prevention of clinical neuropathy. We propose the following four steps:

1. Subclinical stage I, which may be called stage of involvement, can be considered as characterized by an inflammatory reaction within the nerve trunk ("neuritis") but as yet without either subjective or objective manifestations. However, it may be assumed that during this stage the immunopathological mechanism has been triggered.

2. Subclinical stage II, which may be called the stage of nerve damage. We consider that this stage may be accompanied or not by subjective clinical manifestations such as pain or tenderness, but loss of function is absent. Manifestation of pain seems to be related to the rate and kind of nerve fiber degeneration ([2]). This stage may not be associated with pain and tenderness in the so called "silent neuropathy" (we have previously proposed the expression of "silently arising clinical neuropathy") ([3]).

3. Clinical stage I, which may be called stage of destruction. This clinical stage may be described as characterized by loss of function, but with possible recovery.

4. Clinical stage II, this is the stage of scarring; recovery is not possible.

Pearson and Ross ([4]) assume that as much as 30% of the nerve fibers have to be destroyed before sensory impairment becomes detectable. In the same line of thought, Weller and Cervos-Navaro ([7]) underline that a large proportion of nerve has been damaged before the appearance of clinically detectable neurological deficit.

We think that prevention of clinical neuropathy (meaning destruction of more than 30% of nerve fibers) has to be addressed at the subclinical stage. How can this early subclinical stage be recognized? Is there any indicator clinical sign contemporary to the subclinical neuropathy? In animal models, Crawford, *et al.* ([1]) have demonstrated