be uniformly dispersed in the media. Clear liquid media can also be prepared by using soluble palmitate. Soluble palmitate does not interfere in spectrophotometric studies as well. It is of further advantage that the water-soluble palmitate complex assures a continuous release of the molecularly dispersed palmitate. This unique way of releasing the active substance results in an improved bioavailability, as is clear by the markedly reduced lag period of oxidation by *M. leprae* and *M. phlei.*

Based on preliminary results, the use of the soluble palmitate complex is highly encouraged for the *in vitro* cultivation trials of *M. leprae* as well as for other biological and metabolic studies.

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Avidin-Biotin Immunoblotting Studies on Reactivity of Leprosy Sera with *Mycobacterium leprae* Antigen

To the Editor:

Mycobacterium leprae, the causative agent of leprosy, has the most complex structure of all the mycobacteria ([3]). Fewer antigenic components in the *M. leprae* extracts have been shown in comparison with other cultivable mycobacteria by gradient gel electrophoresis ([5]). Crossed-immunoelectrophoresis of *M. leprae* sonicate developed using sera from leprosy patients has shown about seven or eight antigenic bands ([7]). However, by immunodiffusion about 11 to 12 antigenic components could be detected ([11]). Immunoblotting studies using sera from pooled lepromatous leprosy (LL) patients have revealed about five antigenic bands in the cell-free extract of *M. leprae* ([1]). In this communication, we report an avidin-biotin immunoblotting for analyzing the *M. leprae* antigenic components using sera of leprosy patients.

Collection of serum. Blood samples for the study were collected from untreated leprosy patients attending Central JALMA Institute for Leprosy (CJIL), Agra, India. Patients were clinically classified on the criteria of Ridley and Jopling ([9]). Blood samples from healthy laboratory volunteers served as controls. Sera were separated and stored at −70°C. Normal healthy sera from a non-endemic region for leprosy were kindly provided by Dr. H. D. Engers, World Health Organization, Switzerland.

***M. leprae* antigen.** The cell-free extract of armadillo-derived, purified *M. leprae* ([2]) was kindly provided by Dr. R. J. W. Rees from the IMMLEP (WHO) bank, London.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE of the *M. leprae* antigens was carried out according to the method of Laemmli ([8]) in 12% homogenous gel along with molecular weight markers

(Pharmacia, Sweden) at a constant current in a discontinuous buffer system using a Pharmacia PAGE apparatus.

Immunoblotting. Immunoblotting was done as per the method of Towbin, *et al.* ([12]). Here the SDS was removed from the electrophoresed gel by soaking it into blotting buffer for ½ hr with two changes. The gel containing *M. leprae* proteins was later blotted onto nitrocellulose paper (S & S, Germany) using a Pharmacia electroblotting apparatus.

Avidin-biotin system. Biotin is covalently coupled to free amino groups of anti-IgG/IgM, and avidin is conjugated to horseradish peroxidase. To each molecule of biotin many molecules of avidin bind by strong noncovalent interaction, thus making the avidin-biotin interaction a convenient and highly sensitive system ([4]).

Enzyme reaction. Strips of about 3-mm breadth were cut from the antigen-containing nitrocellulose paper, and the free sites were blocked with 5% fat-free casein solution [or 3% bovine serum albumin (BSA) solution] in phosphate buffered saline (PBS), pH 7.4. The blocked nitrocellulose strips were then incubated for 2 hr at room temperature in 2 ml casein/BSA solution with 20 μl of different test sera on a horizontal shaker (Rockomat, Switzerland). Strips were then washed five times with PBS and reincubated for 2 hr in 2 ml of casein/PBS solution with 1:1000 antihuman biotinylated IgG (Sigma, U.S.A.). The strips were washed again five times, and were incubated for 2 hr more in 2 ml of casein/PBS with peroxidase-conjugated avidin (1:1000). The strips were washed again five times, and were incubated in α-4 chloronaphthol (Sigma, U.S.A.) substrate solution for about ½ hr, or until the contrasting reaction was observed. The strips were then rinsed in deionized water, dried, and photographed immediately. An enzyme reaction was also developed with peroxidase-conjugated antihuman IgG (conventional system) under similar conditions.

In the avidin-biotin system the number of antigens detected in the cell-free extracts of *M. leprae* using sera from different types of leprosy were as follows: 12–14 antigenic bands by using lepromatous (LL) sera; 12 using borderline lepromatous (BL) sera; 10 using midborderline (BB) sera; 5 using borderline tuberculoid (BT) sera; and 1 using tuberculoid (TT) sera (Fig. 1).

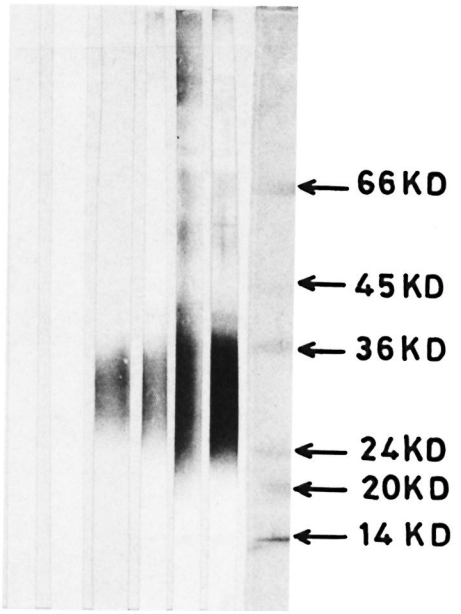

FIG. 1. Immunoblotting of *M. leprae* antigens on nitrocellulose paper and reaction with serum samples using the conventional developing technique: Lane 1 = control serum; lane 2 = TT serum; lane 3 = BT serum; lane 4 = BB serum; lane 5 = BL serum; lane 6 = LL serum; lane 7 = molecular weight markers.

In a conventional system (without avidin-biotin), the number of antigens detected using sera from different types of leprosy were as follows: 10 using LL, 10 using BL and 4 using BB sera. There was no reactivity using TT sera; only one antigenic component could be detected using BT sera (Fig. 2).

Reactivity to the 65-kDa component was observed using BT, BB, BL and LL types of leprosy sera as seen by the avidin-biotin assay. However, it could hardly be detected in TT, BT, and BB sera by conventional blotting procedures. Reactivity to the 60-kDa and 48-kDa proteins was observed in only BB, BL and LL sera by the avidin-biotin assay, and in only BL and LL sera by the conventional assay.

Reactivity to the 30–40-kDa component was observed in all of the leprosy patients by the avidin-biotin assay. However, by the conventional assay the reactivity was not seen with TT leprosy sera.

In the present study as many as 12–14 antigen-reacting components could be seen

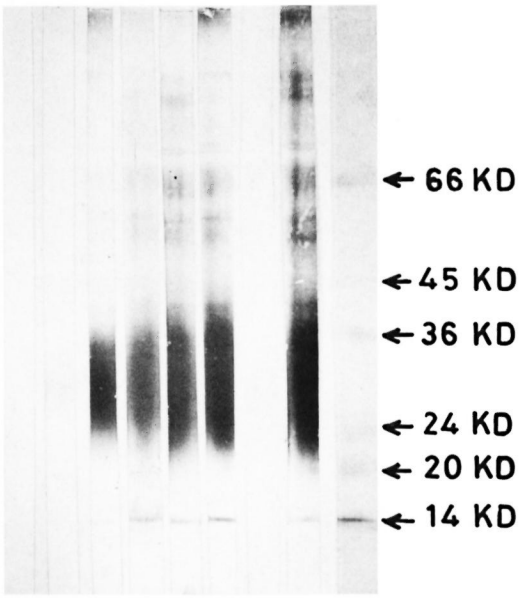

FIG. 2. Immunoblotting of *M. leprae* antigens on nitrocellulose paper and reaction with serum samples using the avidin-biotin technique: Lane 1 = control serum; lane 2 = TT serum; lane 3 = BT serum; lane 4 = BB serum; lane 5 = BL serum; lane 6 = LL serum; lane 7 = LL pooled serum absorbed with *M. leprae*; lane 8 = pooled LL serum absorbed with *Escherichia coli*; lane 9 = molecular weight markers.

using LL sera by the avidin-biotin assay in comparison with five components in pooled LL sera as shown by autoradiography ([1]) and about 10 components by conventional immunoblotting. Klatser, *et al.* ([6]) could not identify *M. leprae* antigen using sera from tuberculoid cases. In the present study using the avidin-biotin system, BT sera could identify as many as five antigenic components. By the SDS-polyacrylamide gel electrophoresis immunoperoxidase technique (SGIP) about 12 antigenic components are reported in the cell-free extract of *M. leprae,* but the assay is cumbersome, delicate, and difficult to reproduce.

In the present study the identification of the number of antigens was higher, as expected, by LL sera than by BT sera. However, minor variations in the identification of a number of antigens were observed within the same groups. The reactivity to the 65-kDa component of *M. leprae* was noted in most of the leprosy sera tested by the avidin-biotin system. This protein has been reported to be ubiquitous since it could be found in all of the bacteria ([10]).

The detection of a higher number of antigen-reacting components in the present study could be attributed to the augmented sensitivity of the assay using the avidin-biotin system.

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Autoradiographic Evidence of Reduced Epidermal Cell Proliferation in Leprosy Patients

TO THE EDITOR:

Leprosy is an infectious disease having unique peculiarities among which are an early and preferential peripheral nerve impairment and a continuous spectrum of clinical manifestations between two poles, tuberculoid and lepromatous. The changes in nerves ([2, 4–7, 9, 16, 22]) and the dermal inflammatory process ([3, 8, 11, 13, 17, 28]) have been studied using ultrastructural, immunocytochemical and cell kinetic methods. However, epidermal changes scarcely have been studied ([11, 13, 14, 21]). Mitsuda ([18, 19]) suggested that the differences in the local response to leprosy antigens could be due to the resistance of neuromacular leprosy patients as opposed to nodular leprosy patients. It is now known that it is the state of the cellular immunity of the patient that determines the appearance of a specific clinical manifestation of the disease ([10]). Thus, at the tuberculoid pole the patient has lesions with a high ratio of T4 (helper)/T8 (suppressor) lymphocytes and with levels of interleukins 1 and 2 and of interferon-gamma which allow him to present an inflammatory response with cells able to kill the bacillus. At the lepromatous pole are those patients whose macrophages are just hosts to the bacillus ([13]). The number of Langerhans' cells and keratinocytes as well as the Ia+ phenotypic expression by keratinocytes also correlate with the clinical and morphologic spectrum of leprosy ([10, 12–15, 26, 27]). One of the most important clinical features of this disease is the occurrence of trophic ulcers. Although it has been suggested that they might be caused by cutaneous anesthesia, decreased epidermal proliferation could also be their cause.

In the present communication, preliminary quantitative data on epidermal cell proliferation determined by biopsies of cutaneous lesions of leprosy patients, using tritiated thymidine as a marker, are reported.

Leprosy patients, newly diagnosed and untreated, 10 to 60 years old, of either sex, clinically classified as tuberculoid (TT), borderline tuberculoid (BT), indeterminate (Ind), borderline lepromatous (BL), and lepromatous (LL), received clear and careful explanations about the purpose of the study and the procedure to be used. After giving their written consent, they received a 0.1 ml ^{3}H-thymidine (100 μCi/local, Sp. Act. 6.7 Ci/mol; Dupont, U.S.A.) intradermal injection at a site showing a cutaneous anesthetic lesion and at an unaffected contralateral site. The injection sites were either on the trunk or limbs. One hr later, 4.0-mm punch biopsies were made at both injection sites. The whole procedure was always performed during the morning.

The biopsy fragments were fixed in 10% formaldehyde, containing 10% glycerine, for