

## Fluorescent Staining for *Mycobacterium leprae* in Tissue Sections

### Comparison with Fite-Faraco Procedure<sup>1</sup>

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Fluorescence microscopy for detecting mycobacteria was first used by Hagemann (7) in 1937. Further publications are rare. This method is employed more commonly in smears (1, 2, 4, 5, 8, 12) and rarely in tissue sections (9, 11). It gives better results in comparison with the Ziehl-Neelsen stain in both types of material. In sections of lesions due to leprosy, fluorescence microscopy has not been used to detect bacilli, except for Mansfield (11) who observed that with this method bacilli were easily and rapidly found. On the other hand, the Faraco (3) and Fite-Faraco (6) modifications of the Ziehl-Neelsen stain for leprosy bacilli in tissue sections give better results than the original Ziehl-Neelsen technic.

#### MATERIALS AND METHODS

Skin biopsies of 30 cases of leprosy were studied. The lesions presented rare or no bacilli with the Fite-Faraco technic. The forms of leprosy were indeterminate, tuberculoid and lepromatous, the latter being in a resolving stage (Table 1). As controls, typical lepromatous cases were used. From each case, ten paraffin sections, 6  $\mu$  thick, were made, all being attached to slides without albumin. From these, five sections were stained by the Fite-Faraco technic (6) and five for fluorescence microscopy (10), using alternate sections for each procedure. For fluorescence microscopy, auramine and rhodamine were used for staining as recommended by Kuper and May (10), differentiation was done with 0.5% aqueous HCl and dehydration in absolute alcohol. Clearing in xylol was omitted. Slides and coverslips were of good quality, free of scratches and cleaned by immersion in concentrated nitric acid for 48 hours, then washed in tap and distilled water. Mi-

croscopic examination of the slides was done within 24 hours following staining by the Fite-Faraco and fluorescent procedures.

On evaluating bacillary positivity only typical bacilli were taken into account, i.e., bacilli presenting characteristic form and size and highly yellow fluorescence. Fluorescent material that was too small, too large, rounded or irregular in form, or without features of bacilli was not taken into account.

#### RESULTS

Results are shown in Table 1. Of a total of 30 cases of leprosy, 26 were positive (86.6%) by the Fite-Faraco technic and 10 (33.3%) by fluorescence microscopy.

#### DISCUSSION

The results show that fluorescence microscopy is less advantageous than the Fite-Faraco technic for finding leprosy bacilli in tissue sections, the positivity being 33.3% by the former, and 86.6% by the latter technic. Fragmented bacilli or bacillary dust were not taken into account because of the difficulty in distinguishing them from artifacts. This high positivity with the Fite-Faraco technic may be due to the improvement and accuracy of the method, revealing a greater number of bacilli (3), but it was not surpassed by fluorescence microscopy. It is possible that in comparison with routine Ziehl-Neelsen procedure the latter may be more advantageous for finding leprosy bacilli as it is in smears and tissue sections for tubercle bacilli (1, 2, 4, 5, 8, 9, 11, 12).

It is not our purpose to demonstrate the value of Faraco or Fite-Faraco method comparative to the Ziehl-Neelsen technic. This has been done previously (3). The former was more advantageous, revealing a greater number of bacilli. Leprosy cases with rare or no likely bacilli were chosen because these cases are more suitable and significant for purpose of comparison than others with many bacilli (Figs. 1 and 2).

<sup>1</sup>Received for publication 29 December 1972.

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TABLE I. *Leprosy bacilli in tissue sections: fluorescence microscopy versus Fite-Faraco procedure.*

Leprosy forms	Total no. cases	Fite-Faraco procedure		Fluorescence microscopy	
		Positive	Negative	Positive	Negative
Indeterminate	5	4	1	0	5
Tuberculoid	16	13	3	6	10
Lepromatous	6	6	0	4	2
Tuberculoid (reactive stage)	3	3	0	0	3
Total	30	26 (86.6%)	4 (13.3%)	10 (33.3%)	20 (66.6%)

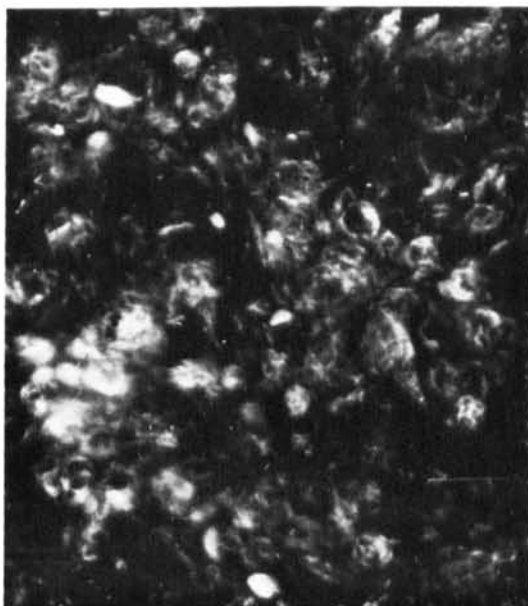


FIG. 1. Lepromatous leprosy with numerous bacilli. Fluorescence microscopy. X 544.

The high frequency of artifacts present in sections stained for fluorescence microscopy is emphasized. These artifacts present a fluorescence similar to that of leprosy bacilli. Such artifacts were produced by phenol used in preparing the staining solution and by albumin used to attach sections on the slides. The artifacts due to albumin were avoided but not those due to phenol, because stain without the latter is poorly preserved. In distinguishing artifacts from bacilli it is important to take into account the morphology of the bacilli.



FIG. 2. Tuberculoid leprosy in reactive stage, a short bacillus. Fluorescence microscopy. X 1360.

### SUMMARY

Leprosy bacilli in tissue sections were stained for fluorescence microscopy. Thirty cases of leprosy with few bacilli were studied. Bacillary positivity was less with this method (33.3%) than with Fite-Faraco procedure (86.6%).

## RESUMEN

Se tiñeron bacilos de lepra en cortes de tejido para microscopía fluorescente. Se estudiaron treinta casos de lepra con pocos bacilos. La positividad bacilar fué menor con este método (33.3%) que con el procedimiento de Fite-Faraco (86.6%).

## RÉSUMÉ

Dans des coupes de tissu, on a coloré des bacilles de la lèpre par des méthodes de microscopie en fluorescence. Trente cas de lèpre ont été étudiés, qui ne présentaient que peu de bacilles. La positivité bacillaire était moindre par cette méthode (33 pour cent), qu'avec le procédé de Fite-Faraco (86,6 pour cent).

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