DEMONSTRATION OF MYCOBACTERIUM LEPRAE IN SECTIONS
IN 532 CASES OF LEPROSY

COMPARATIVE STUDY BETWEEN THE ZIEHL-KLINGMULLER
AND THE WADE-FITE TECHNIQUES

R. D. AZULAY, M.D. AND LUCIA M. C. ANDRADE, M.D.
Laboratory of Pathologic Anatomy, Institute of Leprology
National Leprosy Service, Rio de Janeiro, Brazil

Those who are accustomed to routine histopathological work in leprosy
are aware of the difficulties of demonstrating M. leprae in sections by the
usual methods of acid-fast staining. The difficulty of this demonstration
may be increased by the effects of sulfone treatment. In the past several
workers (1-5) attempted to improve the technique. In an article with
Portugal (7) we showed the value of the Gram-Weigert method in this
matter.

There is no doubt that the acid-fastness of the organisms of the genus
Mycobacterium depends upon the presence of lipidic substances in its
constitution. It is also a fact that a large part of these lipidic substances
are extracted by such reagents as alcohol and xylol used in routine histo­
logical technique; this fact accounts for the partial or total loss of the
acid-fastness of M. leprae when paraffin sections are used. As has re­
cently been pointed out by Wade (8), in an article which should be read
by those interested in this matter, there are two fundamental principles
by which these difficulties may be overcome.

1. The principle of restoring the acid-fastness of the organisms. This
principle was involved in a completely new technique published in 1938
by Faraco (1). This depends on refatting the germs after they have been
rendered nonacid-fast in the imbedding and deparaffinizing sequences, by
heating them with some substance such as a vegetable oil, or chicken fat,
or even lubricating oil before staining them with fuchsin. While attempt­
ing to improve this technique, Fite, Cambre and Turner (4) hit upon a
method which Lillie (6) calls the "Fite-Faraco" method. The appropri­
ateness of this name is denied by Wade, as will be seen.

2. The principle of protecting the acid-fastness of the organisms. In
the article referred to, Wade claims priority for this principle, one which
he had employed for over 25 years but had not previously published. He
points out that when the bacilli are subjected to the influence of any of
the reagents used in clearing the tissues in the imbedding process, they
are made liable to ("conditioned for") lipid extraction when they are
again treated with a fat solvent in removing the paraffin. To avoid that

1 Presented at the VI International Congress of Leprology, Madrid, October 1958,
in the Portuguese language. Translated, with certain modifications approved by the
authors.
sequence ("double jeopardy") he originally used essential oils throughout
the process—bergamot or origanum oil for deparaffinizing and anise oil
for clearing the sections after staining.

This method is cumbersome and has been abandoned by Wade in favor
of what he regards as an improvement of the one devised by Fite and
associates (4). He holds that that method is not one of refatting but of
protection; the sections are deparaffinized by a mixture of a paraffin
solvent and an oil, the latter protecting the bacilli while the wax is being
removed. Consequently the term "Fite-Faraco" is a misnomer. His own
improvement, the "Wade-Fite" method, is given in detail below: 2

In order to ascertain the value of this new technique we decided to
undertake a comparative study of it with the Ziehl-Klingmuller method
used routinely in this laboratory.

MATERIAL AND TECHNIQUE

The material studied consisted of 532 skin specimens from sulphone-treated and
untreated patients. These specimens had been fixed in 10 per cent formal in physio-
logical serum and imbedded in paraffin by the usual histological process. The staining
techniques as we used them are as follows:

I. ZIEHL-KLINGMULLER TECHNIQUE

1. Xylene, two times until completely deparaffinized.
2. Alcohol sequence, 100%, 90%, 70%; wash in tap water.
3. Carbol-fuchsin, 25 to 30 minutes, at room temperature; wash.
4. Decolorize with 5% sulphuric acid; wash.
5. Stain with Carrazzi hematoxylin, 5-10 minutes.
6. Water, 10 minutes.
7. Alcohol sequence, 70%, 90%, 100%.
8. Clear with xylene.
9. Mount with Canada balsam.

II. WADE-FITE TECHNIQUE

1. Deparaffinise with a mixture of equal parts of liquid petrolatum
   and rectified turpentine.
2. Blot with filter paper until of semi-dry appearance.
3. Wash with tap water, 5 minutes.
4. Carbol-fuchsin, 25 to 30 minutes, at room temperature; wash.
5. Blot with filter paper. 2
6. Decolorize with 20% sulphuric acid; wash.
7. Stain with Carrazzi hematoxylin, 5-10 minutes. 3
8. Water, 10 minutes.
9. Blot with filter paper and place in an incubator at 56° to dry. 4
10. Mount with Canada balsam. 3

2 In the same article Wade described the use of a water-soluble imbedding wax, a
mixture of solid polyethylene glycols called Carbowax by the manufacturer, by which
the use of fat solvents is avoided entirely, but that has nothing to do with the present
investigation.

3 In these steps we departed from the original technique as published by Wade, who
does not blot before decolorizing, (b) specifies dilute Loeffler's methylene blue for
counterstaining, (c) allows the sections to dry at room temperature, and (d) mounts
in any of the modern synthetic resins, which cause less rapid fading than does
Canada balsam.
RESULTS

The findings in the comparison of duplicate sections stained by these two methods are given in Table 1. Statistical study of the figures there given shows that the differences are significant.

<table>
<thead>
<tr>
<th>Findings</th>
<th>Number of cases</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both sections negative</td>
<td>237</td>
<td>44.6</td>
</tr>
<tr>
<td>Both positive and quantitatively equal</td>
<td>80</td>
<td>15.0</td>
</tr>
<tr>
<td>Both positive, more bacilli in W-F</td>
<td>115</td>
<td>21.5</td>
</tr>
<tr>
<td>Both positive, more bacilli in Z-K</td>
<td>9</td>
<td>1.7</td>
</tr>
<tr>
<td>W-F positive, Z-K negative</td>
<td>88</td>
<td>16.5</td>
</tr>
<tr>
<td>Z-K positive, W-F negative</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>Totals</td>
<td>532</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The most interesting feature of these findings is the number of specimens which are found positive by the Wade-Fite technique but negative by the ordinary Ziehl-Klingmuller method. We repeated the examination two or more times with 19 of these 88 specimens and obtained positive results with the Ziehl-Klingmuller method in only three instances, although one which was Wade-Fite positive in the first examination was negative in the later ones.

Detailed study of these 88 cases showed: (a) Two which were 3+ with W-F and negative with Z-K were, on repetition, 1+ and 2+, respectively, with Z-K.

(b) Six were 2+ with W-F but negative with Z-K. One of these, on repetition, was 1+ with Z-K. Another changed from 2+ with W-F to 3+ with that method but remained negative with Z-K. It is to be noted that the original smear of the latter case, made from the biopsy specimen and stained by Ziehl-Gabbett, was positive.

(c) Eighty were 1+ with W-F and negative with Z-K. Smears from the biopsy specimens had been made in 38 of these cases, and 9 were positive (1+) while the other 29 were negative.

Referring to the 3 cases which, as shown in the table, were found to be positive with Z-K (1+) but negative with W-F, the following was found on repetition:

(a) One became positive (1+) with W-F.

(b) One gave the same results as before.
One had revealed only very rare acid-fast bacilli in one small nerve
twig found in the Z–K section.
Mention may be made of the marked difference in size of the two
groups of cases in which the bacilli were more numerous in one section
than the other. This was the case in no less than 115 cases—more than
20 per cent of the whole—with the W–F method but only 9 cases with
the Z–K method.

SUMMARY AND CONCLUSIONS

The authors have made a comparative study of two methods of stain­
ing M. leprae in paraffin sections of biopsy specimens of the skin, namely,
the Ziehl-Klingmüller method and the Wade-Fite in which, according to
Wade, the bacilli are protected from extraction of their lipids during the
deparaffinization. The data of the findings show differences which are
statistically significant, and the following conclusions are drawn.
1. That the Wade-Fite method of staining gives better results in
demonstrating M. leprae than does the Ziehl-Klingmüller method.
2. Repeated examinations of a number of specimens showed that in
most cases the results remained the same, thus demonstrating that posi­
tivity actually depends on factors inherent in the method itself.
3. The fact that there were 38 cases found positive (1+) with the
Wade-Fite method but negative with the other, of which only 9 were
positive in smears made from the biopsy specimens, suggests that the
new technique is not exclusively one of protection of the acid-fastness of
M. leprae in the lesions but that it also has the property of restoring acid­
fastness which has been lost in the tissues.

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