NOTES

Microculture Morphology of Mycobacteria

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Morphology continues to be indispensable for primary isolation and recognition of bacteria. Under controlled conditions of growth, microscopic and gross characteristics of most bacterial species are constant, replicable, and have high differential values. This report is concerned with microculture morphology of Mycobacterium tuberculosis and four atypical groups which were classified by standard methods, as recommended by a committee of the American Thoracic Society (Am. Rev. Respirat. Diseases 87:459, 1963).

A smooth suspension of a young culture, matching a no. 1 MacFarland density standard diluted to 0.25, was the inoculating source. Two 5-mm loopfuls were planted on 7H10 agar plates. Six planting sites were previously conditioned by roughing the agar surface. A cover glass (13 by 13 mm) was then placed over each planted area. The plates were incubated in Mylar plastic bags at 37°C. At 24-hr intervals for 6 days, a cover glass was removed, inverted, and sealed with a nonfluorescent mounting medium to a standard microslide. After heat fixation, slides were stained in phenol-auramine and quenched in 10% aqueous ferric chloride (S. W. Gilkerson and O. Kanner, J. Bacteriol. 86:890, 1963). A 200-w HBO lamp and a blue-light, orange-yellow filter system with a bright-field condenser provided adequate illumination for binocular observation.

Laboratory cultures and recent isolates of Runyon's groups I (M. kansasii), II, III, and IV (M. fortuitum) were included in the study. M. tuberculosis strain H37RV and a recent isolate of M. tuberculosis served as a morphology baseline.
Group differences were determined by micro-colony morphology, cell size, fluorescence, cording, beading, and branching.

Rough cultures developed well-formed cords by mid-cycle (Fig. 1). These included the H37RV strain, the recent isolate of *M. tuberculosis*, a very rough culture of *M. kansasii*, and two rough cultures of *M. fortuitum*. Distinction among rough cultures based on cord morphology could not be made. All cultures of groups II and III were smooth and noncorded.

In 48 hr, smooth cultures of *M. kansasii* developed loosely organized microcolonies of long, beaded bacilli (Fig. 2). At 96 hr the microcolonies had spread into large noncorded masses of fine, filamentous bacilli (Fig. 3). At this stage, beading was markedly reduced, and the bacilli were individually less distinct.

Group III produced microcultures of nonbeaded bacilli with rapid changes in size as a chief characteristic (Fig. 4). At planting time, the bacilli were very short, 1.2 μ (Fig. 5). In 48 hr they increased their size seven times and developed branches (Fig. 6). At 96 hr, most of the long forms had reverted to coccoidlike bacilli (Fig. 7). Twelve cultures, classified as group III by routine methods, duplicated closely the cultural changes of *M. kansasii*. However, all attempts to induce them to grow as photochromogens, including cultures from infected mice, failed. After repeated tests by standard methods, they retained the characteristics of group III, and by microculture they continued to show the micromorphology of *M. kansasii*. It seems reasonable to suggest that these cultures represent nonpigmenting variants of *M. kansasii*.

Smooth cultures of *M. fortuitum* formed microcolonies with dense centers of bacilli. The central masses were surrounded by large lacelike halos of bacilli (Fig. 8). In areas where microcolonies were well isolated, this pattern continued through the test period, but closely adjacent colonies coalesced into smooth masses.

Seventeen cultures repeatedly run through the gamut of standard tests developed skotochromogens and other cultural reactions which placed them in group II, but they had no group identity by microculture. Thirteen produced microculture morphology consistent with group III; the other four grew the micromorphology of smooth *M. kansasii*.

Grouping by standard methods of the atypical cultures in this study and the relationship to grouping by microculture are presented in Table 1.

The foregoing experiments showed that Mycobacteria formed four types of micromorphology: one for each of Runyon's groups I, II, III, and IV (smooth cultures), and one for the cording process of *M. tuberculosis* and rough cultures of groups I and IV. These became distinctively differential within 96 hr. Also, all cultures of group II and some cultures of group III fit the micromorphology of other groups (Table 1). These morphological types were replicable.

This technique serves well for early detection of growth of clinical specimens, as well as for a valuable aid to standard methods for rapid grouping. From work now in progress, it appears certain that it can be used for direct susceptibility to antituberculous drugs, and for the

![Figure 4](http://jb.asm.org/)

**Figure 4.** Average length of group III bacilli, T-37 culture, at 24-hr intervals. These changes were typical of 37 cultures classified by routine methods as group III.

**Table 1.** Relationship of standard grouping (Runyon) and microculture morphology grouping of 81 atypical cultures

<table>
<thead>
<tr>
<th>Smooth cultures</th>
<th>Standard grouping</th>
<th>Microculture morphology grouping</th>
<th>Microculture morphology fitting other groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Mycobacterium kansasii)</td>
<td>13</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>17</td>
<td>0</td>
<td>13 in group III, 4 in group I</td>
</tr>
<tr>
<td>Group III</td>
<td>49</td>
<td>37</td>
<td>12 in group I</td>
</tr>
<tr>
<td>Group IV (M. fortuitum)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>52</td>
<td>29</td>
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</tbody>
</table>
recognition of mixed infections of *M. tuberculosis* and a smooth, atypical form. These can be accomplished by microscopic examination without culture purification.

Fig. 5. Size of T-37 culture at planting time. X 500.
Fig. 6. T-37 culture at 48 hr. X 500.
Fig. 7. T-37 culture at 96 hr. X 500.
Fig. 8. Microcolony of *Mycobacterium fortuitum*, O-41 culture, at 48 hr (O = Oteen isolate). X 500.