Effect of Egg Yolk on Growth of *Mycobacterium tuberculosis* in 7H12 Liquid Medium

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Of 92 drug-resistant *Mycobacterium tuberculosis* strains isolated from sputum specimens, 86 showed growth in two types of 7H12 broth, one with egg yolk and the other without egg yolk. In addition, two strains grew only in plain 7H12 broth without yolk, and four others were recovered only in the medium supplemented with egg yolk. The radiometrically detected growth was higher in the presence of egg yolk, corresponding to a higher number of CFU per milliliter in these cultures. The improvement of growth in 7H12 broth supplemented with egg yolk was most noticeable in cultures isolated from sputum specimens having a low number of acid-fast bacilli in the smear and producing only a few colonies on solid media.

It was reported recently (7) that the addition of egg yolk to 7H12 broth does not interfere with the radiometric detection of growth in this medium. The aim of our study was to determine whether the addition of egg yolk to 7H12 broth would enhance the growth of *Mycobacterium tuberculosis* cultures isolated from specimens obtained from patients undergoing chemotherapy and having a drug-resistant bacterial population.

**Media.** Standard 7H12 broth (Johnston Laboratories, Inc., Towson, Md.) in 4.0-ml vials was used in this study. The 50% egg yolk enrichment solution manufactured by Difco Laboratories, Detroit, Mich., was added at a volume of 0.1 ml per 7H12 broth vial before inoculation. Two types of 7H12 broth, one with and one without egg yolk, were supplemented with PANTA, a drug combination manufactured by Johnston Laboratories to prevent contamination, which contains polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. Lowenstein-Jensen slants were obtained from BBL Microbiology Systems, Cockeysville, Md. 7H11 plain and selective agar media (Middlebrook and Cohn 7H11 agar base; BBL) were prepared in our laboratory in biplates. The selective agar contained polymyxin B, amphotericin B, carbencillin, and trimethoprim (4, 5).

**Procedure.** Sputum specimens were processed by the conventional *N*-acetyl-l-cysteine sodium hydroxide method (6). After digestion-decontamination, the pellet was suspended in a 0.2% solution of bovine albumin fraction V, and five media were inoculated: 0.5 ml each of the 7H12 vials, 0.2 ml per each side of the solid medium biplate containing 7H11 plain and 7H11 selective agar media, and 0.1 ml per each Lowenstein-Jensen slant. The growth in 7H12 vials was recorded daily in the BACTEC 460 instrument (Johnston Laboratories), and growth on solid media was checked weekly.

**Radiometric detection of growth in 7H12 broth with and without egg yolk.** From a total of 92 isolated strains, 86 cultures were recovered in both types of 7H12 broth, 2 others were recovered in plain 7H12 broth only, and the remaining 4 were recovered solely in 7H12 broth with egg yolk (Table 1). The mean times of radiometric detection of positive initial growth (growth index [GI], >20) were 11.5 and 11.8 days for the plain and yolk-containing media, respectively. The GIs during the subsequent period of observation were significantly higher in the presence of egg yolk (Table 2). For this analysis we selected only those 64 cultures in which the GI in one or both of the 7H12 vials reached the maximum of 999. An average of 15.8 days was required to achieve GI 999 in either one or both vials; a longer period (23.9 days) was required for cultures isolated from smear-negative specimens and from specimens containing a low number of acid-fast bacilli (AFB; less than 50 AFB per 50 fields) or a low number of recovered CFU (less than 10 colonies). However, the mean time to achieve GI 999 was 12.2 days for cultures with a higher number of AFB in the smear or of colonies on solid media or both (Table 2). A cumulative GI value has been suggested for evaluation of the effects of different supplements on growth in 7H12 broth (1). The mean cumulative GI for 64 cultures in 7H12 broth with egg yolk was 1.74 times higher than for cultures in plain 7H12 broth. The difference in mean cumulative GI was even more impressive (2.39 times) for cultures recovered from specimens with a low number of AFB or CFU or both (Table 2).

**Growth curves in 7H12 broth with and without egg yolk.** First experiments were conducted to determine the number of CFU per milliliter in 7H12 broth with and without egg yolk. Growth curves based on these data were compared with the radiometric GI daily-reading curves. An example of such comparisons (strain Vertullo) is presented in Fig. 1. This strain is resistant to all conventional antituberculosis drugs and is used in our laboratory for quality control of drug susceptibility tests. The difference in the GI curves (Fig. 1A) is typical of most of the cultures described above: the daily GI readings were higher in the presence of the egg yolks, and the maximum on the GI scale was reached in this medium a few days before occurrence of the maximum in the yolk-free medium. The actual growth curves (Fig. 1B) show the same phenomenon for the yolk-containing and yolk-free 7H12 broth cultures. The number of CFU per milliliter at the end of the exponential phase of growth in the presence of egg yolk was about 10 times higher than that in yolk-free medium. In experiments with two other strains, the difference was only three- to fourfold: 4.2 × 10⁷ versus 1.0 × 10⁷ with one of the cultures, and 3.7 × 10⁷ versus 1.2 × 10⁷ with the other. In experiments with two remaining strains, the number of CFU per milliliter at the end of the exponential phase (6.5 × 10⁷ for one of these strains and 1.0 × 10⁷ for the other) was the same with or without egg yolk. Probably the

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TABLE 1. Growth of M. tuberculosis from sputum specimens in 7H12 broth

<table>
<thead>
<tr>
<th>Smear result (AFB/field)</th>
<th>Total no. of isolated cultures</th>
<th>No. showing growth in 7H12 broth</th>
<th>With yolk</th>
<th>Without yolk</th>
<th>With and without yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34</td>
<td>1</td>
<td>0</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> A total of 5 to 40 AFB per 50 fields.

differences obtained with different strains in these experiments are a reflection of the fact that 7H12 broth provides more favorable conditions for some strains than for others. Generally, the maximum number of CFU per milliliter observed in these experiments, i.e., between 10<sup>6</sup> and 10<sup>7</sup> in yolk-free medium, corresponds with that observed in our previous studies of the growth curves of M. tuberculosis in 7H12 broth (2, 3). It seems that for certain strains, the bacterial multiplication can, in the presence of egg yolk, lead to a higher number of CFU per milliliter. In experiments with three strains, for which the number of CFU per milliliter in the presence of egg yolk was higher than the number in the cultures without egg yolks, the differences in the shapes of GI curves in the presence and absence of egg yolk were as shown in Fig. 1. There were no differences in GI curves in the presence and absence of egg yolk for those two strains for which the maximum number of CFU per milliliter was the same under both conditions of cultivation. This fact is probably an indication that the differences in the shape of GI curves and a higher cumulative GI in the presence of the egg yolk, observed for 50 of the 64 strains described above, were a reflection of the fact that the number of CFU per milliliter was higher in the presence of the egg yolk. For other strains, lack of difference in the GI curves (14 of the 64 analyzed in Table 2) or generally slow growth under both conditions (22 other strains) was probably an indication of no significant difference in the number of CFU per milliliter, regardless of the presence or absence of egg yolk.

Discussion. In experiments with 92 drug-resistant M. tuberculosis cultures, we found that the addition of egg yolk to 7H12 broth improved the extent of growth for the majority of the strains tested, although the average rate of detection of initial growth (GI, >20) was the same. The more important fact is that in the egg yolk-containing medium, the GI values were higher than those in plain medium (Fig. 2). This was especially advantageous for specimens having a low number of AFB in the smear or producing a low number of colonies on solid media inoculated with these specimens. Lack of difference, in the presence or absence of egg yolk, in the number of days required for detection of the initial growth (GI, >20) is probably due to the utilization of nutrient substances from the egg yolk, which may compete with the 14C-labeled palmitic acid. In some cases (Fig. 1), we did observe higher GIs in cultures supplemented with egg yolk, even at the beginning of cultivation, which may be an indication that competition between egg yolk and the radio-labeled source of carbon was at least not significant. Better growth detected radiometrically corresponded with a higher number of CFU per milliliter in the presence of egg yolk.

Good growth in cultures inoculated with specimens from tuberculosis patients undergoing chemotherapy is essential for subsequent drug susceptibility testing, an important step in the management of tuberculosis. Poor growth of the isolate often results in a loss of viability of the culture during subcultivations. Therefore, the rapid detection of the initial growth in 7H12 broth in the BACTEC system solves only a part of the problem. The introduction of the egg yolk supplement makes 7H12 broth not only a medium of rapid detection, but also a medium for obtaining well-grown M.

TABLE 2. Cumulative growth of M. tuberculosis from sputum specimens in 7H12 broth with and without egg yolk

<table>
<thead>
<tr>
<th>AFB and/or CFU contents</th>
<th>No. of specimens</th>
<th>Mean no. of days required to achieve GI 999</th>
<th>Mean cumulative GI ± SD</th>
<th>Statistical significance</th>
<th>Relative coefficient of growth (a/b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With yolk (a)</td>
<td>Without yolk (b)</td>
<td>t</td>
<td>p</td>
</tr>
<tr>
<td>Low</td>
<td>20</td>
<td>23.9</td>
<td>2,404 ± 783</td>
<td>1,006 ± 705</td>
<td>5.95</td>
</tr>
<tr>
<td>Moderate and high</td>
<td>44</td>
<td>12.2</td>
<td>1,849 ± 708</td>
<td>1,152 ± 732</td>
<td>4.56</td>
</tr>
<tr>
<td>All specimens</td>
<td>64</td>
<td>15.8</td>
<td>1,931 ± 667</td>
<td>1,107 ± 721</td>
<td>6.70</td>
</tr>
</tbody>
</table>

FIG. 1. Daily GI readings (A) and growth curves (B) for M. tuberculosis Vertulio in 7H12 broth with (○) and without (●) egg yolk.
tuberculosis cultures. This may be important for subsequent use of 7H12 cultures as sources for the NAP (para-nitro-α-acetyl-amino-β-hydroxy-propiophenone) test, identification, and drug susceptibility testing. In general, the egg yolk supplement should be considered an important tool for recovery of M. tuberculosis from specimens obtained from patients undergoing chemotherapy, when the emergence of drug-resistant, poorly growing cultures can be expected. Because of the observations presented in this paper, for primary isolation of M. tuberculosis from clinical specimens obtained from patients undergoing chemotherapy, we now use, along with three solid media, only one vial of 7H12, supplemented with egg yolk.

LITERATURE CITED