VII. COMPARISON OF BLOOD AGAR-PENICILLIN AND LOWENSTEIN-JENSEN MEDIA UNDER ROUTINE DIAGNOSTIC CONDITIONS

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In recent studies (Tarshis, 1952, 1953) it was found that a modified human blood medium was more satisfactory for the cultivation of tubercle bacilli than several other blood media previously described (Tarshis and Frisch, 1951; Frisch and Tarshis, 1951). Since comparison of this medium under routine diagnostic condition was somewhat limited, a more thorough evaluation was felt desirable. In this investigation the blood medium was compared with the Lowenstein-Jensen medium (Holm and Lester, 1941, 1947).

MATERIALS AND METHODS

The formulas and the methods of preparing the media are given below.

Blood medium. Agar,¹ 1.5 g; glycerol (analytical grade), 1.0 ml; human bank blood (with ACD solution, see below), 25.0 ml; water (distilled), 74.0 ml; penicillin (50 units per ml)²; final pH approximately 6.8.³

The agar was dissolved in the glycerol-water by heating. The mixture was sterilized by autoclaving at 15 pounds pressure (121 C) for 15 minutes, after which it was cooled to 45 C in a water bath. The blood and penicillin were added, the ingredients thoroughly mixed, and approximately 3.5 ml of medium were dispensed aseptically into sterile 16 by 125 mm screw-cap tubes, and allowed to harden into slants. The flask was kept in a beaker of water in the bath to prevent tipping when the medium became depleted during the tubing process and was shaken frequently to maintain thorough mixture. Outdated human bank blood approximately four weeks old was used and contained ACD solution as follows: Citric acid, 0.50 g; sodium citrate, 1.37 g; and glucose, 2.45 g per each 100 ml of transfusion solution. Approximately 500 ml of blood were collected in Baxter Transfuso Vac bottles containing 120 ml of ACD solution. To obviate possible inhibitory effects of any single blood, several specimens of the same type were pooled before use. However, as yet this has not been found to be a problem.

Lowenstein-Jensen medium. Monopotassium phosphate, 2.4 g; magnesium sulfate, 0.24 g; magnesium citrate, 0.6 g; asparagine, 3.6 g; glycerol, 12.0 ml; distilled water, 600.0 ml; potato flour, 30.0 g; homogenized fresh whole eggs, 1,000.0 ml; malachite green (2 per cent aqueous solution), 20.0 ml.

The salts were dissolved in the glycerol-water by heating in a 56 C water bath, and the potato flour was added in small portions with constant stirring. Heating and stirring were continued until the mixture assumed a cooked appearance. Next the eggs were washed thoroughly with soap, water, and alcohol, then dried, cracked, and the contents homogenized aseptically by means of a Waring blender. Finally, the required amounts of egg and malachite green were added to the salt-potato flour mixture and the ingredients mixed well. Approximately 3.5 ml of medium were dispensed aseptically into sterile 16 by 125 mm screw cap tubes. The medium was coagulated and sterilized by inspissation at 90 C for 1 hour. All media were checked for sterility and then stored in the refrigerator before use.

¹ Retort Pharmaceutical Co., Inc., Long Island City 1, New York, Lot no. 17Y.
² Crystalline penicillin G obtained from various manufacturers. Aqueous preparations of the antibiotic increase the pH approximately 0.01 to 0.1 of a pH unit.
³ Determined by the Beckman pH meter.

Seventy-two hour sputum specimens from tuberculous patients were used for study. The sputa were concentrated by the sodium hydroxide method (Tarshis and Lewis, 1949), and two tubes of each medium were inoculated with equal quantities of sediment from each specimen. The tubes were incubated at 37°C in a nearly horizontal position to permit uniform distribution of the inocula. All tubes were capped loosely for varying periods of time to permit evaporation of excess moisture and then tightened to prevent drying of the media. Readings were made every other day under a bright light for a period of eight weeks. Stained preparations of questionable colonies were examined microscopically.

RESULTS

A summary of the results is presented in table 1.6 It will be noted that of 1,012 specimens, 378 were positive. Of these, 93.6 per cent were obtained on the blood medium as compared to 89.9 per cent on the Lowenstein-Jensen medium, or a difference of 3.7 per cent, which is not significant. Thirty-eight strains of tubercle bacilli grew on the blood medium but failed to do so on the Lowenstein-Jensen, and 24 strains that grew on the latter medium failed to develop on the former.

Table 1

**Comparison of blood agar-penicillin and Lowenstein-Jensen media under routine diagnostic conditions**

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Lowenstein-Jensen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent</td>
</tr>
<tr>
<td></td>
<td>1-7</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>15-21</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**D. Comparison of contamination**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of tubes of each medium</td>
<td>2,024</td>
<td></td>
</tr>
<tr>
<td>Number of tubes contaminated, blood</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Number of tubes contaminated, Lowenstein-Jensen</td>
<td>140</td>
<td></td>
</tr>
</tbody>
</table>

The average time of first appearance of growth on the blood medium was 18.9 days as compared to 21.5 days on the Lowenstein-Jensen medium, or a difference of 2.6 days less required for growth on the former medium.

During the first week 2.3 per cent of the specimens were positive on the blood medium as compared to 0.3 per cent on the Lowenstein-Jensen. After 3 weeks the number of positive findings on both media were more nearly equal, showing 71.8 per cent positive cultures on the former medium and 66.8 per cent on the latter.

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6 Method of reporting adopted from article by Melvin et al. (1951).
The contamination rate was slightly less on the blood medium than on the Lowenstein-Jensen. Of 2,024 tubes of each medium, 102 of the blood were contaminated (5.0 per cent) as compared to 140 tubes of the Lowenstein-Jensen (6.9 per cent) which is not significant.

Although not included in this report, it should be pointed out that during the past year approximately 300 tuberculous exudates from various lesions were cultured on the same two media under the same conditions and essentially similar results were obtained as with the sputum specimens.

DISCUSSION

As pointed out previously (Tarshis and Frisch, 1951; Frisch and Tarshis, 1951; Tarshis, 1953), four distinct types of mature colonies of virulent human tubercle bacilli have been observed thus far on the blood media. These are shown in figure 1. When first noted they are pinpoint in size, light gray, glistening, and easily recognized against the red background of the blood when examined under a bright light. They gradually increase in size and reach maximum growth in about 5 to 6 weeks. Colonies are usually typical within 2 weeks. When mature they vary greatly in diameter from approximately 1 to 10 mm and assume a tannish-gray or red color, but some may become dark tan with a slight greenish cast. Other colonies may acquire varying combinations of these colors. Depending upon the size of the inoculum, growth on the blood medium, in some instances, can be detected as early as 3 to 4 days.

When contamination occurs, growth is usually confluent and nearly always takes place within 24 to 48 hours. The medium also turns dark and in some cases almost black. Uncontaminated

![Figure 1](http://jb.asm.org/)

**Figure 1.** Typical types of mature colonies of virulent human tubercle bacilli observed on the blood agar-penicillin medium.

a. Irregular, warty, or cauliflower-like colony.

b. Irregular, warty, pyramidal colony with nipple-like apex.

c. Slightly different form of colony type b with a more distinct nipple-like apex.

d. Irregular, warty, doughnut shaped colony.

e. Small, smooth, dry, convex colony of various geometric shapes with little tendency to obliterate the boundary lines. Occurs almost invariably on medium with heavy growth.

In this as well as previous studies (Frisch and Tarshis, 1951; Tarshis, 1953) it has been noted that a number of strains of tubercle bacilli grow on one type of medium but not on another. It has been observed also that certain dysgonic mycobacteria grow better on blood than egg media. This again emphasizes the cultural variability of some strains of M. tuberculosis, and a maximum investigated further for its value in the determination of the susceptibility of tubercle bacilli to other antibiotic and chemotherapeutic agents.

SUMMARY

In the study described it has been demonstrated that a modified human blood medium containing agar, glycerol, blood, and penicillin was satisfactory for the cultivation of tubercle bacilli under routine diagnostic conditions.

Figure 2. Different stages in the development of a freshly isolated virulent human strain of Mycobacterium tuberculosis at 37 C on the blood agar-penicillin medium.

a. After 4 days' growth. When first noted colonies are pinpoint in size, light gray, glistening, and easily recognized against the red background of blood when examined under a bright light.

b. After 14 days' growth. Colonies are very much larger, darker gray, dry, and usually quite typical at this stage of development. Both smooth, convex, and slightly irregular, warty colonies may be seen.

c. After 21 days' growth. Colonies are still larger and have assumed a more distinct irregular, warty morphology.

d. After 42 days' growth. Colonies have reached maximum development and measure approximately 5 to 7 mm in diameter.

Efficiency for isolation is not likely to be achieved by the use of a single medium. For this reason, and because of its efficiency, it is felt that the blood medium should be included with others for routine diagnostic work. It is believed also that it would be the medium of choice for the small laboratory in which the culture for tubercle bacilli is limited by the difficulties in preparing or procuring the more elaborate and expensive media.

In a study in progress it has been found that the medium is very satisfactory for determining the susceptibility of tubercle bacilli to streptomycin and isoniazid (isonicotinic acid hydrazide), an important procedure in therapy. Since it fulfills the criteria of an ideal medium for this purpose and also because it compares favorably with other media in current use, it should be in-

In comparison with the Lowenstein-Jensen medium the results were essentially the same. The number of positive cultures obtained and the average time of the first appearance of growth were slightly better on the blood medium than on the Lowenstein-Jensen medium. The number of tubes contaminated was slightly less for the former than the latter medium.

The chief advantages of the blood medium are economy, simplicity of preparation, and ability to grow tubercle bacilli from small inocula easily, recognizably, and in a short time. The ingredients are readily obtainable, and fresh media can be prepared in a few minutes. Refrigerated media have proved exceedingly stable in tightly sealed screw cap tubes for as long as four months. All these factors should encourage a wider use of
cultural methods for the bacteriologic diagnosis of tuberculosis.

REFERENCES


