TECHNICAL METHODS

A Simple and Safe Method for Determining the Sensitivity of Tubercle Bacilli to Antituberculous Agents

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(RECEIVED FOR PUBLICATION SEPTEMBER 9, 1957)

Chemotherapy of tuberculosis, to be effective, demands adequate laboratory control of antituberculous drugs. It is, therefore, important to establish a convenient, reliable, and rapid method of handling large numbers of sensitivity tests *in vitro*.

The method to be described employs a composite medium for primary isolation of the tubercle bacillus. This medium consists of a Löwenstein-Jensen (L.J.) slope incorporating in the bottom of the tube 2 ml. of modified Besredka (1921) liquid egg yolk medium.

Modified Besredka Medium

The yolks of two eggs are beaten up and added to 200 ml. of distilled water. It is important that all egg white be removed. After thorough shaking, the mixture is clarified by adding to it 1% NaOH (75 to 80 ml. should be sufficient). The clarified mixture is then filtered through Chardin paper and the volume of the filtrate made up to 600 ml. with distilled water. The pH is adjusted to 8.0 with N/20 HCl, after which the medium is bottled in 100 ml. amounts. Sterilization is carried out by tyndallization. Just before the final steaming, 0.5 ml. of 2% malachite green is added to each 100 ml. amount. For use, the medium is distributed aseptically in 2 ml. amounts into the bottom of L.J. slopes.

Procedure

Specimens after decontamination and concentration are inoculated into Löwenstein-Jensen-Besredka (L.J.B.) slopes, which are incubated horizontally at 37° C. for one week. They are then set upright and incubation continued for six weeks before being discarded as negative. Growth is usually apparent on the surface of the medium in 11 to 21 days. Growth characteristics are identical with those obtained on standard L.J. slopes.

Sensitivity Testing

The fluid portion of the primary culture is used as the inoculum for L.J. slopes in which are incorporated the appropriate concentrations of streptomycin, isonicotinic acid hydrazide (isoniazid), and

FIG. 1.—Four consecutive drops of the Besredka portion of a primary L.J.B. culture of tubercle bacilli have been delivered from a pasteur pipette and allowed to run down the surface of L.J. medium. The uniformity of inoculum attainable by this means is indicated by the identical growths resulting after 26 days' incubation at 37° C.

p-aminosalicylic acid (P.A.S.). An L.J. slope with no added antituberculous agent is inoculated for each strain under test as a growth control.

For inoculation of test media, one drop of the Besredka growth from the primary culture is allowed to run down the surface of each of the test and control slopes, using a Pasteur pipette fitted with a rubber teat. The tests are incubated at 37° C. for 21 to 28 days, after which results are read. A sensitivity test is accepted as readable only if the growth control tube contains over 20 colonies or a confluent growth. This ensures that an adequate inoculum has been made into each tube under test because, as shown in Fig. 1, consecutive drops of Besredka primary culture from the same pipette produce identical growths on fresh L.J. medium.

Discussion

Determination of the sensitivity of strains of tubercle bacilli to antituberculous drugs, especially where large numbers of specimens are handled, can be laborious, time-consuming, and dangerous to the operator. The method described has been adopted in order to minimize these drawbacks. It is essentially a solid medium method of sensitivity testing, and is, therefore, suitable for use with streptomycin (Stewart,

1955), isoniazid, and P.A.S. (Medical Research Council, 1953). The culture media and techniques employed are within the resources of most bacteriological laboratories. Primary growths of tubercle bacilli on L.J.B. medium are readily recognizable and this medium has been found to be as satisfactory as L.J. medium for isolating the organisms from sputum concentrates. The fluid portion of a primary culture, when used in the manner described, provides, for all practical purposes, uniform inocula for the test and control slopes of sensitivity tests, thereby obviating recourse to a second subculture with its attendant delay and potential inaccuracies (Mitchison, 1953). Risks to technicians from mechanical emulsification of tubercle bacilli are also avoided. The simplicity of the method enables large numbers of sensitivity tests to be performed rapidly (approximately 24 per technician per hour) so that the extent to which different concentrations of test substances are used need virtually be limited only by available incubator space. The method has proved itself convenient to use in a general laboratory dealing with some 3,000 sensitivity tests per annum. It has provided results which clinical colleagues have found to be consistent and reliable.

Summary

A simple, safe, and efficient method is described for determining the sensitivity of tubercle bacilli to standard antituberculous agents.

I wish to thank Dr. A. J. O'Hea, Department of Bacteriology, University of Glasgow, for advice and criticism.

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Paper Electrophoresis of Urinary Porphyrins

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(RECEIVED FOR PUBLICATION MAY 24, 1957)

A simple method is described for the isolation and the determination of the different porphyrins excreted in urine in cases of porphyria.

Twenty-four hours' urine is collected with toluene as preservative and 500 ml. is taken for the extraction of the porphyrins. The urine is acidified with glacial acetic acid to pH 4.0 and left for 24 hours covered with a layer of toluene for the separation of the porphyrins. The porphyrins separate as a brown precipitate, which partly floats in the urine. The precipitation of porphyrin is not complete, but a small amount which precipitates much more slowly was found by electrophoresis to have the same composition as the mixture which comes down in the first 24 hours' standing. The urine is filtered through Whatman No. 42 filter paper. The precipitate on the filter paper is washed with distilled water till the washings are neutral to litmus. Barbitone buffer (10.3 g. of sodium diethyl barbituric acid and 1.84 g. of diethyl barbituric acid in 1 litre of water) is prepared and a minimum volume of this buffer (about 5 to 8 ml.) is poured on the filter paper over and over again to dissolve the porphyrins. The precipitate dissolves completely giving a deep red solution. This solution is used for the separation of the porphyrins by paper electrophoresis.

The inverted "V" type of electrophoresis apparatus (Flynn and de Mayo, 1951) is used for the electrophoresis with Whatman 3MM filter paper and the same barbitone buffer used to dissolve the porphyrins. The porphyrin solution is applied to the middle of the dry Whatman 3MM filter paper in the same way as the blood serum is applied and the paper is fixed in the electrophoresis chamber with the two ends dipping in the buffer solution compartments. The buffer solution is allowed to soak up the filter paper from both ends so as to meet in the middle where the porphyrin solution has been applied.

The power supply is switched on when the whole paper is soaked with the buffer solution. About three to four hours' run under the same conditions as are used for the electrophoresis of serum proteins gives about 9 to 10 cm. separation of the porphyrins. After being dried in a hot air oven at about 60 to 70° C., the filter paper is examined in ultra-violet light and photographed to obtain a permanent record. A Hanovia-Muir table model ultra-violet lamp fitted with a 500-watt straight arc tube and