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

NINAN VERGHESE

*From the Department of Biochemistry,
 Madras Medical College, Madras, India*

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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

FIG. 1.—Urinary porphyrins photographed in ultra-violet light after paper electrophoresis from a case of porphyria.

FIG. 2.—Pattern of porphyrin excretion in urine in two cases.

fluorescence filter giving ultra-violet light around 3,200 A.U. at 40 in. distance is used as the source. The separated porphyrin fractions show beautiful red fluorescence, and the area and the intensity of the fluorescence give an approximate relative proportion of the individual porphyrin fractions excreted in the urine (Figs. 1 and 2). For identification, purified porphyrins can be spotted as markers and run parallel with the porphyrin mixture extracted from the urine.

A characteristic electrophoretic pattern of porphyrin excretion can be obtained by this method and will be useful in comparing the porphyrin excretion in the same individual periodically, and also for studying the pattern of porphyrin excretion in cases of porphyrinuria and porphyria.

I am grateful to Dr. A. R. Natarajan, Chemical Examiner to the Government, and Mr. R. Viraraghavan for helping me to study and photograph the porphyrins in ultra-violet rays.

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A Container for Handling and Centrifuging Pathological Material

C. B. FISHER AND A. J. HOGAN

From the Department of Pathology and Bacteriology, Welsh National School of Medicine, Cardiff

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For many years workers in pathology laboratories have used the "universal" container for the collection and handling of pathological material.

Although the "universal" container has proved to be a most useful bottle, it has the following serious drawbacks:

(1) Whitwell, Taylor, and Oliver (1957) found that when infected fluid in a "universal" container is spun in an angle centrifuge some of the material is sprayed over a wide area around the centrifuge, the size of the area being about 7 ft. in diameter.

If the containers are spun in a "swing-out" type of centrifuge the buckets and the outsides of the containers may be heavily contaminated, though neither the inside of the centrifuge casing nor the atmosphere is contaminated.

(2) Should at any time the cap be faulty or not properly tightened, material may escape and contaminate the outside of the bottle during normal handling.

(3) When material with a low cell content is centrifuged, cells are deposited across the whole of the floor of the bottle, and are then very difficult to recover.

In an attempt to overcome these faults, a bottle has been designed with a trough around the neck and an inner bottom surface which is conical (Fig. 1).

The bottle is made of transparent "bottle" glass, with a diameter of base $1\frac{1}{2}$ in. and overall height of $3\frac{1}{2}$ in. The neck is threaded to take the standard 1 oz. bottle metal cap with rubber liner, so as to allow the rim of the cap to come below the edge of the trough wall. The depth of the trough around the

FIG. 1.—Narrow-neck container.