TECHNICAL METHODS

THE BACTERIOLOGICAL DIAGNOSIS OF TUBERCULOUS MENINGITIS

BY

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With the introduction of streptomycin and other drugs for the treatment of tuberculous meningitis and the subsequent finding that the outcome of the treatment depends upon therapy being begun early (Illingworth, 1950), the diagnosis of tuberculous meningitis has become a clinical and laboratory emergency procedure. Since many conditions can produce a cellular and biochemical picture in the cerebrospinal fluid similar to that found in tuberculous meningitis, the essential laboratory procedure lies in the immediate and accurate demonstration of tubercle bacilli in the fluid.

It was stressed by Cathie and MacFarlane (1950) and in the Ministry of Health Report on Streptomycin Trials (1950) that tubercle bacilli could be found in the direct films of a large percentage of cases if the necessary care were taken in the preparation of the film. Unfortunately, technical methods are still being advocated (Cruickshank, 1951; Harvey, 1952) which, if generally applied, will result in a failure in many cases to demonstrate the organisms. It would therefore seem of some use to record a reliable method for the preparation of such films.

The most important steps in any method for the identification of tubercle bacilli in the cerebrospinal fluid by direct film would seem to be as follows: (1) The containers and slides to be free from acid-fast material; (2) the collection of as large a volume of cerebrospinal fluid as is clinically possible; (3) adequate centrifugation; (4) the removal of all but a few drops of the supernatant fluid to ensure that such organisms as are present are contained in the minimum of fluid necessary for the emulsification of the deposit; (5) efficient emulsification of the deposit; (6) the minimum film

area; (7) light counter staining to allow identification of organisms lying on the cells; (8) examination of the whole film.

The method described below has been in use at the Children's Hospital, Sheffield, for three and a half years.

Method

Cerebrospinal fluid, 10–20 ml., is collected in a new, clean, sterile 1-oz. container. After the fluid has been well mixed 9 drops are removed with a capillary pipette for the cell count and the remainder is centrifuged at 2,500 revolutions per minute for 30 minutes. The supernatant fluid is removed for biochemical examinations with a capillary pipette, leaving a volume equivalent to 3 or 4 drops in the bottle. The deposit is emulsified in the fluid remaining in the bottle, care being taken to mix up all the material from the base of the container. A clean slide is placed across the angle of the tripod and a drop of the suspended material is put in the centre. A Bunsen burner is placed near the slide and the edges are allowed to dry to prevent the spread of the fluid over the slide. The remaining fluid from the bottle is then placed on the top of the first drop and the whole film dried. The diameter of the film is kept down to 5 to 7 mm. unless the cell count of the original cerebrospinal fluid is of the order of 400 to 600 per c.mm., when a diameter of up to 1 cm. is more satisfactory. The film is stained with Ziehl-Neelsen's carbol fuchsin and decolorized in the usual way. The counter stain (malachite green has been used in this series) is only applied for 20 seconds and the slide is then washed well with tap water and blotted dry. The whole smear is examined using the 1/12-in. objective. This examination takes from half an hour to one and a half hours.

Sufficient deposit remains in the container to allow a film for Gram's stain and cultures for pyogenic organisms to be made after the addition of a small quantity of sterile normal physiological saline.

The whole of the first specimen is used for the preparation of the smear and for examination for

pyogenic organisms. The specimen taken off at the beginning of intrathecal streptomycin therapy can conveniently be used for culture on Löwenstein-Jensen medium and for guinea-pig inoculation.

Comment

Using this method acid-alcohol-fast bacilli were found in the cerebrospinal fluids from 91 out of the last consecutive 100 cases of tuberculous meningitis. All were subsequently confirmed by culture and/or guinea-pig inoculation.

The method was carried out with the usual routine laboratory apparatus and without any special training. The results reported include those

obtained by resident clinical pathologists and technicians.

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