FIG. 3.—Diagram of embedding box with the "serial blocks" of an appendix in position, before the box is filled with paraffin wax. On the right is shown an appendix after removal from molten wax, straightened out before being cut into serial blocks.

This "serial block" method of embedding has been used successfully in various investigations. Cooper and Daniel (1949) and Cooper, Daniel, and Whitteridge (1951, 1955) used it in a study of muscle spindles in extrinsic eye muscles. In this work many of the "serial blocks" had serial sections cut from them, so that the whole length of the muscle could be studied in a set of sections which had taken perhaps a tenth of the usual time to cut, mount, and stain. The method is also valuable for the study of the whole brain or brain-stem of small animals, and was used successfully by Bosanquet, Daniel, and Vollum (1953) in a study of the exudate produced by intrathecal tuberculin in the guinea-pig. The spinal cord, hypothalamus, pituitary stalk and pituitary, the vermiform appendix, lengths of peripheral nerve, and lengths of arteries have also been examined by this method.

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The Filtration of C.S.F. in the Bacteriological Diagnosis of Tuberculous Meningitis

BY

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The early detection of tubercle bacilli in the C.S.F. is an urgent and time-consuming occupation, and the earlier removal to hospital of suspected cases of tuberculous meningitis only makes the bacteriological problem more acute. The present report deals with an attempt to improve the chances of an early positive laboratory diagnosis by ensuring that all bacilli in the fluid are available for examination. This is of importance where there are only a few bacilli per millilitre of fluid and when, as Silverstolpe (1949) noted, many of the organisms do not in fact deposit on centrifugation. The centrifugal filter described by Elek and Hilson (1951) appeared to offer a convenient unit for making the total bacterial content of the specimen readily available on the plane removable surface of a collodion membrane.

Experimental Methods

Each specimen of 5 to 15 ml. of C.S.F. from suspected cases was divided equally as soon as possible after withdrawal from the patient (to obviate clotting), half being treated in (a) the routine fashion, i.e., centrifuging at 3,000 r.p.m. for 30 minutes, removing the supernatant, and using the deposit for building up films and for culture, and half being submitted to (b) filtration. Two to 7 ml. of C.S.F. was filtered through a membrane of A.P.D. 0.6 μ , five to seven minutes at 1,500 to 2,000 r.p.m. sufficing in most cases to complete the operation. After filtration the membrane was treated in a variety of ways to obtain satisfactory films, and whole or part of it was placed face-upward on a Löwenstein-Jensen slope of the same batch as that used in (a) above. The routine film and culture (a) was thus a control on the experiments under consideration.

Results

Of 18 specimens of C.S.F. examined, 13 were bacteriologically proven tuberculous, four were bacteriologically and clinically non-tuberculous, and one was bacteriologically negative but strongly suspect on clinical grounds as tuberculous.

Direct Films

Table I compares the results of methods (a) routine and (b) filtration. Five of 13 undoubted positive cases were positive by method (a), whereas only one

TABLE I
DISTRIBUTION OF RESULTS OF DIRECT FILM AND
CULTURE BY ROUTINE AND FILTRATION TECHNIQUES

		Direct Film		Culture	
		+	-	+	-
Routine (a)	..	5	12	6	12
Filtration (b)	..	1	16	13	5

was positive by method (b). In no case was an unequivocal positive found in (b) where not also present in (a). By both methods a very protracted search was made for acid-fast organisms; in two instances these were only found by method (a) after two and a half and three hours. A number of positives showed very pronounced beading of the bacilli, in one case giving the picture of a short-chain streptococcus; occasionally by method (b) acid-fast granules were found, but though these were suggestive, and were not found in cases subsequently deemed non-tuberculous, a firm diagnosis could not be made.

A variety of methods was used in an attempt to improve filtration results: 1, rubbing the membrane face-downward on a sterile glass slide (this gave the best films, preserved the cell picture, and was used in the majority of the cases reported); 2, scraping or shaking up the membrane in a minimal amount of saline or C.S.F. filtrate. Films resulting from these attempts were not as thick as those of method (a). Judging from the cell picture and the finding of organisms, it is possible that the vigorous treatment of the membrane may have disrupted the bacilli to give the coccal forms referred to above. 3, Further experiments, however, suggest that cellulose acetate filters ("oxid") can be used exactly as those of collodion. They are more easily prepared and sterilized, moreover films can be stained *in situ*, dehydrated in alcohol, cleared in xylene, and mounted in the usual manner. Owing to the shortage of suitable specimens, it has not been possible to compare this method with those already reported.

Culture

Table I shows that all 13 cultures were positive by method (b), whereas only six were positive by method (a). There were no positives by the routine technique (a) which were not also positive on filtration (b). The average time of appearance of diagnostically visible growth in the six specimens which grew by both methods was 23 days (limits of 15 to 28 days) by method (a) and 16 days (limits of 10 to 21 days) by method (b), the difference being seven days in favour of filtration. The mean time for growth of those specimens which failed to grow by the routine technique (seven) was 23 days (limits of 17 to 30 days). All cultures were kept for three months before discarding.

The appearance of the tubercle cultures on membranes was striking (Fig. 1), for the collodion takes up

FIG. 1a

FIG. 1b

FIG. 1.—Two examples of colonies of *M. tuberculosis* cultured from C.S.F. on collodion membranes (1.2 cm. diameter).

malachite green, etc., from the medium, becoming very deeply stained, and giving added contrast to the buff-coloured mycobacterial colonies. This contrast, together with the fact that colonies always appear on the disc, simplifies the diagnosis.

The number of bacterial colonies on the membranes varied from one to almost confluent growth. In the single case from which no direct film was made, by method (b) four tubercle colonies only were cultured from 3 ml. of C.S.F. (Fig. 1). In other cases

where films had been made, viable counts would not necessarily be valid.

Discussion

The failure, up to the present, to obtain good films, which was the primary object of the investigation, appears to rule out the method for this purpose unless the third method mentioned above can be made to bear fruit. However, the rapidity of preparation, together with the suggestion of better results on culture, merits further consideration. The method commends itself for the examination of cases under treatment owing to the almost complete removal of inhibitory drugs in the C.S.F. There appears to be a "planting" factor concerned in the growth of *M. tuberculosis*, and this suggests that culture results in specimens other than C.S.F. might be bettered by soaking a dry filter-paper disc in the specimen and applying this to the medium; this method, for different reasons, has been applied by Hoyt, Smith, and Gribkoff (1954) to tubercle culture.

Addendum

Since the work reported was carried out the Hemmings filter* has been introduced. It is possible with a few minor modifications to employ this filter for use with collodion membranes and thus for C.S.F. filtration.

(1) Pressures in the upper and lower chambers must remain the same. An $\frac{1}{8}$ in. hole is bored in the bottom of the upper bijou bottle. The rubber washer sealing the lower container is replaced by one of porous material or with a split aluminium washer.

(2) A thin metal plate with multiple small perforations (1/64 in.) is superimposed over the existing filter plate, in which the holes are too large.

(3) Owing to the requirement of a plane surface thrust on the membrane, which is not given by the curved lips of a bijou, the upper chamber washer must be replaced by two thin rubber washers (0.5–1.0 mm. thick) between which lies a thin metal washer.

Summary

A comparison is made of the results of direct films and culture, by the routine method and by a filtration technique, in the laboratory diagnosis of tuberculous meningitis. In a small series, better results were obtained using the routine method to prepare films, but filtration proved superior in the case of cultures.

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* The Hemmings filter is obtainable from H. A. Jones, 26, Castle Street, Beaumaris, Anglesey.

A Micromethod for the Estimation of Serum Bilirubin

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As a guide in the prognosis and treatment of infants suffering from erythroblastosis foetalis, serial estimations of the serum bilirubin level have assumed greater importance than repeated haemoglobin determinations. This, however, presents a major difficulty. The small amount of blood required for the haemoglobin estimation can readily be obtained by heel puncture, but the quantity needed for the standard bilirubin determinations greatly exceeds that amount, and cannot always be obtained successfully with complete freedom from haemolysis.

The numerous methods for the measurement of serum bilirubin can be broadly classified into two main groups. The first group comprises those methods in which the proteins are precipitated after diazotization of the bilirubin and includes that of King and Coxon (1950). The second consists of those in which protein precipitation is avoided and the bilirubin is diazotized in the presence of catalysts such as urea (Powell, 1944), phenol (Patterson, Swale, and Maggs, 1952), caffeine sodium benzoate (Dangerfield and Finlayson, 1953), sodium benzoate (Jendrassik and Gróf, 1938), and ethanol (Malloy and Evelyn, 1937).

Micro-modifications of the methods of Malloy and Evelyn by Hsia, Hsia, and Gellis (1952) and of Jendrassik and Gróf by With (1943) have been suggested. For both these modifications only 0.1 ml. of serum is used, but a special micro-cell photoelectric instrument is required.

The primary object of this work was to produce a method which would require the minimum quantity of blood and would be simple to perform using standard apparatus available in most laboratories. At the same time, accuracy was required at least equivalent to the more popular methods utilizing quantities of serum up to and exceeding 1 ml. The method of Malloy and Evelyn was selected for modification, as it was the simplest available and most suited for this purpose.

Method

Reagents.—The following were used:

Diazo Reagent

Solution A	Sulphanilic acid	...	...	1 g.
	Concentrated hydrochloric acid	...	...	15 ml.
	Distilled water to	...	...	1,000 ml.
Solution B	Sodium nitrite	...	...	0.5 g.
	Distilled water to	...	...	100 ml.