THE ISOLATION OF *MYCOBACTERIUM TUBERCULOSIS* BY FILTRATION TECHNIQUE FROM CEREBRO SPINAL FLUID

by

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HISTORICAL REVIEW

Isolation of Mycobacterium tuberculosis from cerebro spinal fluid has been always considered the cardinal point for the diagnosis of tuberculous meningitis, although in some instances is difficult or impossible and the diagnosis has to rely on the cytological and chemical changes of the fluid and on the patient's symptoms. To better understand the value of the bacteriological findings and of the pathological changes of cerebro spinal fluid in tuberculous meningitis, it seems advisable to us to review briefly the disease and its new aspects since the advent of therapy.

Tuberculous meningitis is the most common form of tuberculosis of the nervous system. Robert Whytt (124) in 1768 gave the first description of the disease, which he called "acute hydrocepholus". The pathology and clinical symptoms have been subsequently brilliantly described by Trousseau (118), Osler (78), Allbutt (1), Gowers (42, 43), Still (108).

1. Pathogenesis. It is well known that tuberculous meningitis in children usually develops as a complication to a pulmonary primary complex (Wallgren, 121), while in adults is secondary to an extrapulmonary tuberculous lesion (Auerbach, 3). Many theories have been emitted on the pathogenesis of tuberculous meningitis, but only three were supported by facts.

(a) The Hematogenous Theory. Because of the frequent

association of miliary tuberculosis with tuberculous meningitis, it has been thought that meningitis is merely due to an hematogenous spread of tubercle bacilli. Hektoen (47), who in 1896 made a detailed study of the changes of the layers of the cerebral and meningeal vessels, was first led to the conclusion of an hematogenous dissemination of the meninges, having found tuberculous endoarteritis with the formation of intimal tubercles.

(b) The Chorioid Plexus Theory. Kment (56) observed an almost constant involvement of the chorioid plexus in tuberculous meningitis and supposed then that meningitis takes origin in the plexus and that from there is extends to the base of the brain along the periarterial lymph spaces.

(c) The Intracranial Tuberculous Foci Theory. Rich and McCordock (86, 87) first stated that the meninges are not infected by the blood stream, but by a tuberculomata established in the central nervous system during a previous hematogenous dissemination. In other words the cerebro spinal fluid, infected with tubercle bacilli, infects directly the meninges. The following evidence was brought to reject the classical view of meningitis as simply a part of a generalised miliary spread. Miliary tuberculosis and tuberculous meningitis are not always found together. Tubercle bacilli injected experimentally into the blood stream fail to produce meningitis, while they do by direct subarachnoidal injection. In almost all cases of tuberculous

meningitis a tuberculous focus can be found in the brain or meninges, from which the infection spreads into the subarachnoidal space, provided that the search is careful. The authors found such foci in 77 cases out of 82 and this constitutes the main point of their argument. Their findings were contested by Ragins (84), Beres and Meltzer (6). Macgregor and Green (66), McMurray (70), Schmarz (94), agreed with Rich and McCordock that meningitis is commonly caused by tuberculomata, usually localized in the brain substance or in the meninges or, exceptionally, in the adjacent bones, although they believe that this is not the exclusive form of origin. Crowe (28) pointed out tuberculous otitis media as not too uncommon local cause of tuberculous meningitis.

2. Pathology. The tuberculous lesions of the meninges vary considerably in appearance, extent and location. The diffuse, acute inflammatory caseous meningitis (Rich, 87) is characterized by a thick, greenish yellow, often gelatinous exudate in the subarachnoidal space, very abundant usually at the base of the brain. Special investigation on the vascular changes has been done by Hektoen (47), Winkelman and Moore (122), Smith and Daniel (98). They studied carefully the inflammatory modifications of the layers of the vessels of the brain and of the meninges, especially of the arteries. The most remarkable lesions are usually found in the adventitia and in the big vessels,

tubercle bacilli may be demonstrated in great number. The media is less involved and the intima shows subendothelial proliferation. Sometimes the whole vessel wall undergoes complete caseation. In other instances a fibrinoid necrosis is present (Smith and Daniel, 98), with an homogeneous structureless material which involves the subintimal region and partially the media. The lumen of the vessel may be partially occluded by an exudate beneath the fibrinoid material or completely by a thrombus. Smith and Daniel (98) pointed out that the fibrinoid necrosis is not specific for tuberculous meningitis, since they have seen it frequently in the cerebral vessels adjacent to purulent wounds of the brain and Cairns and Russel (12) described it in pneumococcal meningitis. However, whether a true tuberculous degeneration or an aspecific infiltration is present, the important consequence of such vascular changes is the partial or complete obliteration of the lumen of the artery, with an anoxia or infarction of the brain tissue as end result. All the vessels throughout the meninges, the penetrating vessels of the cortex, and especially the arteries of the base and mid-base of the brain, may be involved. The more conspicuous and important the affected artery, the more deleterious the resulting destruction of the corresponding supplied brain area. The first author strongly impressed with the pathological necrosis of the brain tissue in tuberculous meningitis, was Trousseau (118) who, in the

early 1868, made the following statement: "I reject the name of meningitis for cerebral fever, because the lesions of the meninges seem to me to be secondary only, and much inferior in importance to the deep anatomical alterations seated in the brain itself, such as the softening which destroys the fornix, the septum lucidum, the corpus callosum, thalami optici, and posterior part of the cerebral lobes, to a more or less considerable extent. Hence, if the disease should be named after the organic lesions which characterize it, it ought to be called cerebro-meningitis".

Disseminating miliary tubercles can be found, generally not numerous, scattered in the meninges, as hard specks or caseating small nodules. Focal caseous meningeal plaques have been frequently described. They have usually an irregular, flat appearance, varying from few millimeters to one or more cm. in diameter. Very rarely they have been found alone, without a diffuse caseous meningitis. Tuberculomata varying in size, number and activity, have been often seen since Rich and McCordock drew attention to them. They may be found in the cortex, near the ventricles or deeply in the brain substance. Perifocal exudative encephalitis may be present or not (Schwarz, 94). Central necrosis is the most striking feature of active foci and when tuberculomata are very small, caseation is visible only microscopically. Their appearance suggests usually that they are present in the brain before the caseous diffuse meningitis.

In some instances acute, inflammatory caseous meningitis may be confined to a small area, which subsequently becomes organized and an encapsulated, caseous cortical nodule, very adherent to the meninges, has been found as an end result (Rich, 87). It is not known whether the patient's resistance, the pathogenesis in the special case, the number of bacilli or other factors are responsible for a localized caseous meningitis (Schwarz, 94).

3. Age Incidence. Tuberculous meningitis has been regarded as a rare disease in adults, generally secondary to an extrapulmonary tuberculous lesion, where it has been always considered a predominant disease of childhood and adolescence. Wallgren (121) called attention to its development as a complication to a fresh, active primary tuberculosis, especially during the first three months of the disease. The risk diminishes as soon as encapsulation and calcification begin. His observations were confirmed many times by many authors. Although the highest incidence of tuberculous meningitis is still in young people, under 25 years of age (Taylor, 114), in recent years adults have been seen more frequently affected. An explanation to the switch of meningitis towards older people can probably be found in the tuberculization less frequent in children today (Couts, 26), which means that now there is an increase of adults who undergo primary infection and its complications.

4. Clinical Symptoms. The symptoms of tuberculous

meningitis may vary considerably from case to case, according to the age and pleomorphism of the pathological changes of the meninges and of the brain. The onset rarely is acute or abrupt, more frequently there is a prodromal phase which may be protracted for many weeks and which can be overlooked. During this stage intermittent headache is frequent, abdominal pain is present and not uncommonly confused with abdominal syndromes; but the most characteristic symptom is a remarkable modification of the patient's personality, so that adults may be accused of hysteria when the disease is misdiagnosed. In the phase of meningeal irritation stiffness of the neck is present, vomiting frequent and headache intense, due to increased intracranial pressure. As the disease progresses, mental confusion and drowsiness are quite striking, convulsions frequent. When the disease is well developed hydrocephalus is very pronounced, focal signs very pleomorphyc. All kinds of paresis and paralysis have been described, according to the localization and extent of the meningeal and cerebral lesions. In the terminal stage the patient falls in coma, develops very high temperature and dies.

5. Diagnosis. The diagnosis relies on the clinical symptoms and especially on the cerebro spinal fluid findings. Until the advent of streptomycin the diagnosis of tuberculous meningitis was relatively easy, because it relied on a complex of symptoms which appear only late and because the evolution of the disease would have not left doubts about

the etiology, since it was almost invariably fatal. In fact spontaneous recovery has been exceptionally reported (McMahon, 69; Kramer, 58; Macgregor and Green, 66; Smith and Daniel, 98; Lincoln, 62; Choremis and Vrachnos, 16). In the earliest stage of tuberculous meningitis only meningeal irritation may be present and the differential diagnosis with any other mild or uncompletely treated purulent meningitis may be very difficult, for the absence of neurological signs and symptoms. In infants fullness of the fontanella (Smith, 101) may be the only sign and stiffness of the neck may not appear at all. Virus infections as choriolymphocytic meningitis, pre - or non - paralytic poliomyelitis, neurosyphilis and non icteric Weil's disease, brain abscess or tumor have to be ruled out.

6. Cerebro Spinal Fluid Findings Before Treatment. The demonstration of Mycobacterium tuberculosis in the cerebro spinal fluid has been always regarded as the most important finding for certain diagnosis. Although tubercle bacilli should be demonstrated on direct smears in 100 per cent of cases, only few investigators obtained such results (Holt, 53), while others were less successful (Foord, 39; Merritt, 73). Stewart (106) pointed out that the reasons of discrepancy are to be found in the stage of the disease and in the level of the puncture. The chances of finding tubercle bacilli in the cerebro spinal fluid increase with

the advancement of the disease. Cisternal punctures gave him positive smears in 100 per cent of cases within 48 hours of admission, while lumbar only in 57 per cent. Merritt (73) in 1936 made a comprehensive study of the pathological changes of the fluid in tuberculous meningitis. The difficulty in seeing the acid fast bacilli on direct smears, the delay of bacteriological results from guinea pigs inoculation, led him to investigate and to emphasize the cytological and chemical changes of the cerebro spinal fluid for a quick orientation in the diagnosis. "The pressure is elevated (over 200 mm. of cerebro spinal fluid). The appearance is clear or like ground glass, colorless or faintly xantochromic, and the fluid usually shows a delicate weblike clot. There is an increase in white cells (from 25 to 500 per cc. millimeter), lymphocytes predominate. There is an increase in the protein content (from 45 to 500 mg. per 100 cc). The sugar level is decreased (below 45 mg. per 100 cc). The chloride content is decreased (below 650 mg. per 100 cc)."

These findings have been regarded as classical in the advanced stage of tuberculous meningitis, but in the early stage many deviations have been observed. The elevation of portein and cells are usually the most constant findings. However cells may be very numerous, from 1000 to 2000, with predominance of polymorphonucleares (Traeger and Gauthier, 117). Albumino-cytological dissociation may be found in

tuberculous meningitis, making extremely difficult the differential diagnosis with an intracranial tumor. The normal value of glucose (Sedallian, 95) may be a puzzling figure when virus infections have to be excluded. The definite diagnostic importance of low chlorides (Fremont - Smith, 40; Tapie et al, 113) was denied by some authors (Smith et al, 98: Taylor et al, 114), who pointed out that a fall in chlorides appears usually late in the disease and it is partially related to the frequent vomiting.

In conclusion, the chemical and cytological modifications of the cerebro spinal fluid may be typical, but especially in the early stage of the disease, they may be not. The isolation of Mycobacterium tuberculosis remains the most important proof for an unrefutable diagnosis.

7. Treatment of Tuberculous Meningitis. Soon after the discovery of streptomycin (92, 93) and the effectiveness of this drug in inhibiting Mycobacterium tuberculosis in vivo was proved in animals and clinically (37, 38, 48, 125, 126), Cooke, Dunphy and Blake (23) in 1946 reported the first case of tuberculous meningitis in a year old infant, treated with intrathecal streptomycin and cured. This report was followed by many others (8, 19, 24, 27, 30, 49, 50, 51, 57, 67, 79) during 1946-47. After the first enthusiasm for the spectacular effect of streptomycin in a disease considered rapidly and invariably fatal, it was realized that the mortality rate was still elevated,

the relapses frequent, the toxic action of the drug on the nervous system considerable, mainly on the vestibular and acustic centers (McDermott, 68; Stevenson, 105). Intense investigation was pursued to find the factors for an optimal therapy, which would have diminished the mortality rate and allowed complete recovery. The literature on the subject is so voluminous, the schemes of therapy and the conditions of its application so variable not only in different centers, but in a same center for different patients, that any attempt to summarize approaches the impossible. There is only one point of agreement: the necessity of starting the treatment as early as possible. But opinions are still very divided as to what concerns the dosage of streptomycin, the route of its administration, either intramuscular and intrathecal or intramuscular alone, the use of adjuvants, PAS and isoniazid in association with streptomycin or alone, the association of sulfones or of hormones as cortisone or ACTH (2, 7, 9, 10, 11, 13, 14, 15, 17, 20, 21, 22, 25, 32, 33, 34, 35, 36, 41, 52, 54, 55, 60, 63, 64, 65, 71, 72, 75, 76, 77, 80, 82, 85, 88, 89, 90, 91, 97, 99, 100, 102, 104, 109, 110, 112, 114, 116, 119, 120).

However, the main problem of therapy in tuberculous meningitis remains how to let streptomycin to reach tubercle bacilli protected in the thick, fibrinous pockets of the exudate. Cathie (15) first attempted the dissolution of the exudate using a proteolytic enzyme, streptokinase. His promising results were finally indifferent. In 1950 Smith and

Vollum (100) published the first results on the use of intrathecal Protein Purified Derivative of tuberculin (P.P.D.) to dissolve the meningeal exudate. An intense specific inflammatory reaction (111) takes place at the site of the tuberculous lesions after intrathecal P.P.D. administration. Dubos (33) states that proteolytic enzymes supplied by the inflammatory cells may destroy the fibrin or "that the allergic reaction brings about an activation of the normally inactive protease of the serum and that this enzyme can also attack the fibrin". Although many investigators did not accept the introduction of intrathecal P.P.D. administration, the Oxford School (14, 100, 102, 103, 114) supported the resolution of the fibrinous meningeal lesions with good clinical, bacteriological and pathological evidence.

8. Pathology of Tuberculous Meningitis under Streptomycin Treatment. The pathology of tuberculous meningitis, whose detailed classical descriptions were thought not to have left any particular to explore, resuscitated great deal of interest since the modern treatment has been established. It has to be pointed out, however, that we do not know so far which is the pathological substratum of tuberculous meningitis that responded to streptomycin therapy, since all the pathological data have been collected from the fatal cases which did not respond to treatment.

The pathological characteristics in the treated cases

are represented by processes of cicatrization in the brain and in the meninges (Baggenstoss et al., 5; Smith et al., 99), variable in degree and extent, depending on the previous tuberculous lesions, on the length of the disease, on the period of time during which therapy lasted. The paradoxical effect of streptomycin is quite impressive when the drug has been administered in an advanced stage of the disease. Its effect, far away of being therapeutical, will end with a prolonged survival of the patient, during which reparative processes take place, accentuating endoarterial proliferation to such extent that severe stenosis or complete vascular occlusion will occur (Winter, 123; Daniel, 29). Even more striking are the organization and caseation of the meningeal exudate, which is transformed into 1 to 2 cm. thick mass, of woody consistance, with vast areas of caseation, much more in excess than in the untreated cases (Auerbach, 4). These changes will result into the most serious consequences of blockage of the outflow of the cerebro spinal fluid and into very extensive infarctions of the brain tissue.

In contrast to these dramatic changes, the pathological findings of cases treated with intrathecal P.P.D. by the Oxford School (100, 102, 14, 114) deserve special mention. These investigators, in the severe cases that went to autopsy, have not found at the stem of the brain, or highly diminished, the thick, organized exudate which progresses steadily in spite of streptomycin treatment. They

concluded, therefore, that under intrathecal P.P.D. treatment the exudate had dissolved.

9. Cerebro Spinal Fluid Findings During Streptomycin Treatment. Cell count, proteins, go down slowly and progressively during therapy and the patient is considered cured only when these values and the glucose and chloride content are normal and tubercle bacilli no longer present in the cerebro spinal fluid.

A common observation, made during streptomycin intrathecal treatment, are the "peaks" of cells (from 600 to 1600 per cubic millimeter, with prevalent polymorphonucleares) and proteins, usually in the first days of therapy, although they may persist for weeks. Such elevations have been interpreted by the majority of investigators as an irritation due to the intrathecal streptomycin. On the basis of careful bacteriological observation, Smith and Vollum (100) rejected this interpretation. They pointed out that peaks of cells and proteins usually appear when tubercle bacilli are disappearing from the C.S.F. The organisms last generally during the first two or four weeks of therapy. However, an isolated film or culture may be found late in treatment, in which case a spike of cells may accompany the reappearance of the bacilli. Furthermore, these peaks cannot be reproduced by intrathecal injections when the entire disease is controlled. They disappear even though intrathecal streptomycin is being given daily. They are not seen in other meningitis, i.e. Haemophylus influenzae. The authors con-

cluded that such changes in cerebro spinal fluid are specific for tuberculous meningitis and due to the release of tuberculo protein from the dead bacilli. The same phenomena were repeatedly reproduced by injecting intrathecal P.P.D. in sensitized patients without tuberculous meningitis, while they were not in Mantoux negative subjects. These important observations constituted the basis for the introduction of intrathecal P.P.D. treatment combined with streptomycin as a new method of therapy in tuberculous meningitis.

The Oxford investigators (99, 13, 100, 102, 103, 114) have always stressed the importance of bacteriological findings for the diagnosis and evaluation of treatment. In 1954 Smith and Vollum (103) described in detail their technique for the isolation and identification of tubercle bacilli. The sediment of the centrifuged cerebro spinal fluid is used for smears, culture and guinea pig inoculation. Although they did not specify separately the results of their findings in 160 patients, they stated that Mycobacterium tuberculosis may be well isolated by culture or animal inoculation or it may be seen on direct smears if the search is careful.

Harvey (44) reported good results on smears, culture and animal inoculation, centrifuging the cerebro spinal fluid in miniature tubes made by sealing off Pasteur pipettes 1 cm. below the barrel. Out of 150 patients, 76 per cent had positive cultures, 67 per cent positive animals,

74 per cent positive smears, amounting the total number of bacteriologically proved cases to 87 per cent. Stewart (107) using all the sediment from large amounts of fluid, 10 to 20 ml., obtained positive smears in 91 cases out of 100. Excellent results on direct smears (91 per cent of cases positive) were reported also by the Italian school in Florence. Periti and Pasquinucci (81) described a new method to centrifuge cerebro spinal fluid. The fluid, diluted with sterile distilled water, is placed in a special doubled tube with a slide at the bottom, on which the sediment is directly deposited. The slide is easily removed with the outside tube at the end of centrifugation. Muratore and Pasquinucci (76) reported that in 12 per cent of 200 patients tubercle bacilli could only be seen on the smears, while the positive cultures had all corresponding positive smears. They concluded that this method of diagnosis is superior to the isolation on culture, failing tubercle bacilli to grow when still can be seen. They observed that the organisms usually disappear within two weeks from therapy, during which time remarkable changes in morphology occur. They may reappear in case of relapse in great number, before other changes of the fluid take place. Pasquinucci (80) and Galeotti Flori (41) confirmed these bacteriological findings, giving the results of therapy in the same patients. Galeotti Flori pointed out that all the 265 reported cases were bacteriologically proved on smears or culture or guinea pig inoculation.

French investigators, Denhaut et al. (31), Traeger and Gauthier (117) were not so successful with direct smears and do not rely on them for a certain diagnosis. Denhaut et al. (31) compared the results obtained on culture and in animal inoculation for 86 patients. 85 per cent of cases were positive in the animals, 75 per cent on culture, of which 60 per cent were positive on admission and 15 per cent during treatment. In the rapidly fatal cases the cultures did not become negative as soon as treatment was started. In 15 per cent of cases a late culture was positive up to the 30th day of treatment. The authors concluded that animal inoculation is the most sure method of diagnosis, while the culture is more suitable to detect late positive findings. Traeger and Gauthier (117) reported 75 per cent of 68 cases positive on culture and 53 per cent in the animals. They pointed out that patients who responded to treatment became negative quickly, while the fatal cases remained positive. They observed also that a positive culture may be the first sign of relapse, when the other cerebro spinal fluid findings are normal.

Seibold (96) described good results on direct smears, gathering the tubercle bacilli present in cerebro spinal fluid at a cathode by an electrophoretic method. All his eleven patients had positive smears by this method, while for five of them the corresponding cultures were negative.

Since the early days of treatment it was realized that streptomycin interferes with the isolation of tubercle bacilli

from cerebro spinal fluid. Krafchick (57) and McDermott (67) first stressed this effect of the drug to justify the negative bacteriological results during therapy and their explanation was implicitly or explicitly accepted by all investigators as obvious.

Reviewing the literature it is remarkable, with few exceptions, how little has been reported on the bacteriological findings of cerebro spinal fluid during therapy. It is our impression that the short bacteriological information is not due to the fact that investigators are unaware of its importance, but because of the difficulty in obtaining bacteriological evidence.

Levinson (60) recorded on charts the smear and culture results only for three patients out of 19; for the remaining bacteriology is not mentioned. Lincoln and Kirmse (65) did not report the bacteriological findings for 15 out of 18 patients, and for 3 of them, who had a relapse, the following mention is made: "tubercle bacilli fully sensitive to streptomycin were cultured from cerebro spinal fluid of each of the 3 cases during the first week of relapse". Choremis et al. (17) for 29 patients limited their bacteriological information to a + or a - listed in a column headed by "Myco. tuberculosis in the C.S.F." Cathie (15) describing the results in 33 patients, affirmed that "all were bacteriologically proved" with no further specification. Rubie and Mohun (90) proved 87 per cent of 67 patients by culture or animal inoculation; the smears were positive in

65 per cent of cases. Su and Wu (109) reporting 17 cases all bacteriologically proved by smear, culture or guinea pig inoculation, stressed that tubercle bacilli disappeared soon after treatment was started, but in two patients, from whose cerebro spinal fluid the organisms could be recovered until the end of the first month of therapy. Coelho (22) reported 517 patients of tuberculous meningitis in Bombay and pointed out that bacteriological investigation had been impossible because the resources of their laboratories are very limited. Sumner (110) for his 26 cases did not mention bacteriology. Perry (82) stated that in 25 patients out of 26, tubercle bacilli were isolated during life and for the remaining patient at autopsy; further particulars are not given. Ruzickza (91) reporting 114 cases in Vienna, gives the bacteriological results only for the 60 cured patients; 54 out of the 60 were proved to have "tubercle bacilli in the C.S.F. by guinea pig inoculation and histological examination of the animals". Bernard (7) in Argentina, described the results of therapy in 150 tuberculous meningitis and does not mention bacteriology. No mention of bacteriology is also found in Bulkeley (9) for 61 patients; in Torres-Gost (116) for 100 patients. Reley (85) reported 52 cases out of 60, proved on smears or culture and stressed the difficulty in demonstrating tubercle bacilli in cerebro spinal fluid. Waddel (120) had 80 per cent of 25 patients proved on smear, culture or animal inoculation. Robinson (88) reported 72 per cent of 25 cases positive on culture.

When tubercle bacilli are scarce in the specimens, it is difficult to isolate them. A filtration technique has been proved to be effective in collecting the bacilli from various pathological specimens. Using Zsigmondy's (127) membrane filters, Citron (18) isolated tubercle bacilli from urines. From cerebro spinal fluids of tuberculous meningitis patients, Tietz and Heepe (115), Pothmann, Hubert and Wilms (83) stained and demonstrated tubercle bacilli on the membranes. Kunzel (59) cultured Mycobacterium tuberculosis on membrane filters from sputum, pus, puncture fluids. Hawirko and Murray (45, 46) recovered tubercle bacilli from sputa by oil partition and collected them on membrane filters. They demonstrated the bacilli by microscopic examination of the membranes, as well as by culture and animal inoculation.

PURPOSES OF THIS INVESTIGATION

Tubercle bacilli are rather scarce in the cerebro spinal fluid and their isolation has been always difficult. Streptomycin interferes with the recovery of bacilli from the fluid through a direct action on the organisms and through a mechanism of healing process which encapsulates them in connective walls. At the present time tuberculous meningitis is a disease of great interest not only as a diagnostic problem; modern therapy is in a perpetual transformation and still widely opened to research.

Isolation and identification of Mycobacterium tuberculosis is necessary for certain diagnosis, which should not rely exclusively on the demonstration of tubercle bacilli on direct smears. It is essential to determine during treatment the sensitivity to streptomycin or other drugs used, to appreciate the effectiveness of therapy, to establish the correlation between the intrathecal P.P.D. injection and the bacteriological findings. Reliable bacteriological work is also essential for the examination of the clinically recovered patients, in order to establish a bacteriological control of clinical impressions.

Failure to culture Mycobacterium tuberculosis by ordinary methods, led us to attempt the recovery of tubercle bacilli present in the cerebro spinal fluid of tuberculous meningitis patients by a filtration method, using the Zsigmondy's membrane filters.

MATERIAL AND METHODS

From July 1950 up to date, 53 children were admitted to the Alexandra Hospital because of tuberculous meningitis. The patients were given three courses of treatment, with one week rest between each course. They received intrathecal injections of streptomycin daily for 40 consecutive days during the first course of treatment, and every second day for the next 160 days of the second and third course of therapy. Intrathecal P.P.D. (Purified Protein Derivative of tuberculin) was injected every four days when a general and meningeal reaction followed within 24 hours. When no reaction occurred the dosage of P.P.D. was doubled and injected every 2 days, until reaction took place. Cerebro spinal fluids from patients with tuberculous meningitis were sent to the Laboratory before treatment, or at least on admission, and usually every day for the first forty days of therapy; thereafter they were sent every second day for the next hundred and sixty days. From the patients considered clinically cured and discharged from the Hospital, a specimen was received every two weeks for the first two months; then once a month for two months and thereafter once every four months.

At the beginning of this investigation, specimens from 16 patients were cultured directly on Loewenstein medium slopes. Five patients had their cerebro spinal fluid cultured simultaneously by both the filtration method and directly on Loewenstein slopes. Filtration technique was used exclusively as a routine method on 32 patients.

TECHNIQUE

Membrane filters, porosity No. 5, 2 cm. diameter, made by Membrane Filter Gesellschaft, Gottingen, Germany, were used. The membrane, supported by filter paper, was placed in a stainless steel bacteriological filter, and autoclaved at 120°C for 20 minutes. Cerebro spinal fluid was filtered through the membrane using a negative pressure of ca. 300 mm. of mercury. The membrane was planted on Loewenstein medium and incubated at 37°C.

RESULTS AND DISCUSSION

1. Cerebro Spinal Fluids Cultured Directly on Loewenstein Medium Slopes. Cerebro spinal fluids of 16 patients were cultured directly on Loewenstein medium slopes. Of these patients, 12 received 50 milligrams of intrathecal streptomycin every day for the first forty days of treatment and every second day for the next hundred and sixty days. Three patients had 50 milligrams given daily for 40 days and every second day to make up 18 weeks, 6 weeks, 6 weeks, respectively, and thereafter 25 milligrams every second day for the remainder of treatment. For one patient the general scheme was followed with the dose reduced to 25 milligrams throughout his course. Nine out of these 16 patients (see Table I) had been given intrathecal streptomycin for varying times, from one week to eight months, at other hospitals and none of these gave positive cultures on admission or during at least seven months observation and continued treatment. In all but two of these patients, tubercle bacilli were either isolated on culture or demonstrated on direct smear from the cerebro spinal fluids elsewhere before admission. Two of these nine cases died; in one the diagnosis of tuberculous meningitis was proved only at autopsy, while in the other tubercle bacilli had been found elsewhere.

No concentration of the specimen by centrifugation or any other method mentioned on page 16, 17, 18 ? If C.S.F. was used; i.e. not the sediment, then large amounts of SM were present & TB bacilli could not grow.

Seven of the patients (see Table II) were admitted at the onset of tuberculous meningitis before intrathecal treatment was started. Cerebro spinal fluids were cultured directly on Loewenstein medium slopes prior to and after intrathecal

TABLE I

Cerebro Spinal Fluid cultured directly on Loewenstein medium slopes. Investigation on patients transferred to the Alexandra Hospital after intrathecal streptomycin was started elsewhere.

Patient's Name and Age	Positive Findings Reported from Hospitals where the patients were previously treated.		Period of Hospitalization prior to admission to Alexandra Hospital.	Results of investigation carried out at the Alexandra Hospital			
				During I.T. Treatment		After the end of treatment	
	Smear	Culture		Total No. of C.S.F. Tested.	No. of Positive Cultures	Total No. of C.S.F. Tested	No. of Positive Cultures
#1. R.C., 5 years	-	+	2 months	52	0	31	0
#2. J.M.L., 7 years	-	+	4 months	53	0	132	0
#3. G.L., 7 years	+	-	1 week	66	0	1	0
#4. R.F., 3 years	-	-	4 weeks	57	0	20	0
#5. J.F., 14 years	-	-	1 month	4	0 DIED		
#6. V.S., 4 years	+	+	2 weeks	39	0 DIED		
#7. W.K., 6 years	-	+	8 months	77	0	9	0
#8. Y.R., 9 years	-	+	8 months	64	0	21	0
#9. C.M.D., 2 years	-	+	2 weeks	73	0	20	0

- indicates Negative, + indicates Positive, 0 indicates No Growth, C.S.F. indicates Cerebral Spinal Fluid, I.T. indicates Intrathecal.

TABLE II

Cerebral Spinal Fluid cultured directly on Loewenstein medium slopes. Investigation started on patients before intrathecal streptomycin and P.P.D. treatment.

Patient's Name and Age	Before Treatment		During Treatment				After End of Treatment	
	Total No. of C.S.F. Tested.	No. of Positive Cultures	Total No. of C.S.F. Tested.	Positive Cultures			Total No. of C.S.F. Tested.	No. of Positive Cultures
				I week	II week	III week until end		
#10, C.W., 7 years	26	2	44	-	1	-	30	0
#11, F.M., 6 years	1	1	70	-	-	-	30	0
#12, S.L., 6 years	1	1	56	-	-	-	36	0
#13, C.C., 8 years	1	1	49	-	-	-	31	0
#14, B.Y., 5 years	1	1	46	-	-	-	17	0
#15, D.S., 3 years	1	1	76	-	-	-	24	0
#16, B.H., 7 years	2	-	34	-	-	- DIED		

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

streptomycin and P.P.D. treatment. Positive cultures were obtained from all specimens taken before treatment was started, with the exception of one from which we did not isolate tubercle bacilli on any occasion, but which proved tuberculous at autopsy. Mycobacterium tuberculosis was found in a specimen taken during the second week of intrathecal treatment from only one patient.

Tubercle bacilli are often rather scarce in the cerebro spinal fluids and the results obtained by ordinary culture methods were not satisfactory. Out of 16 patients, in only 12 were tubercle bacilli recovered and not beyond the second week of treatment, not even from the patient who received only 25 milligrams doses of intrathecal streptomycin for the duration of therapy. Generally cerebro spinal fluids did not show growth of Mycobacterium tuberculosis as soon as intrathecal streptomycin was started and the average number of isolations was less than two positive per patient.

2. Cerebro Spinal Fluids Cultured Simultaneously on Membrane Filter and Directly on Loewenstein Medium Slopes. Cerebro spinal fluids from five patients (see Table III) were cultured simultaneously on membrane filters and directly on Loewenstein medium slopes. All these patients received 25 milligrams per dose of intrathecal streptomycin. Three of these patients died shortly after admission, but in all five patients the incidence of positive cultures and the number of colonies of tubercle bacilli on the membrane filter was greater than by the direct method on Loewenstein slopes. Before treat-

TABLE III

Cerebro Spinal Fluid cultured on membrane filter and simultaneously on Loewenstein medium directly.

* Patient's Name and Age		Before Treatment	During I Course of Treatment						During II Course of Treatment				Total No. of Positive Cultures
			I week	II week	III week	IV week	V week	VI week	I week	II week	III week	IV week	
#17. M.T., 3 years.													
No.of C.S.F.Tested		2	5	7	6	2	5	3	3	4	3	4	
No.of Positive Cultures.	Membrane Filter	2	4	1	1	-	-	-	-	1	-	1	10
	Loewenstein	2	-	-	-	-	-	-	-	-	-	-	2
#18. M.M., 11 years.													
No. of C.S.F.Tested		1	4	5	4	3	5	2	3	3	2	3	
No. of Positive Cultures.	Membrane Filter	1	2	-	-	-	-	-	-	-	-	-	3
	Loewenstein	1	-	-	-	-	-	-	-	-	-	-	1
#19. M.M., 20 months.													
No.of C.S.F.Tested		1	6	7	6	5	DIED						
No. of Positive Cultures.	Membrane Filter	1	2	1	-	-							4
	Loewenstein	-	-	-	-	-							-
#20. C.T., 2 years.													
No.of C.S.F.Tested		1	1	DIED									
No. of Positive Cultures.	Membrane Filter	1	1										
	Loewenstein	1	-										1
#21. M.B., 3 years.													
No. of C.S.F.Tested		1	3	DIED									
No. of Positive Cultures.	Membrane Filter	0	3										3
	Loewenstein	1	-										1

- indicates No Growth, 0 indicates Not Done, C.S.F. indicates Cerebro Spinal Fluid.

* All received 25 mgm. doses of intrathecal streptomycin.

ment was started the cerebro spinal fluid of one child was cultured directly on loewenstein medium only; the other four had their specimens cultured by both methods. Three of them were positive on membrane filter and direct inoculation on Loewenstein medium, and one on membrane filter only. During treatment all the specimens from the five patients were positive on membrane filter only. In one of the two surviving children no tubercle bacilli could be recovered from the cerebro spinal fluids after the first week of treatment and the other remained consistently positive only on membrane filter until the fourth week of the second course of treatment.

3. Cerebro Spinal Fluids Cultured on Membrane Filter Exclusively. Filtration technique was used as a routine method in the isolation of Mycobacterium tuberculosis from the cerebro spinal fluid of 32 tuberculous meningitis patients. Four of them, under two years of age, received 15 milligrams of intrathecal streptomycin; 28 were given 25 milligrams during all the period of therapy.

(A) Patients who Received 15 Milligrams Per Dose of Intrathecal Streptomycin. Four patients, under two years of age, received 15 milligrams a day of intrathecal streptomycin for the first 40 days of treatment, and every second day for the next 160 days. One of them died, three were discharged cured.

Patient #22, nine months old, who died, was admitted because of miliary tuberculosis. At the Alexandra Hospital

is a routine procedure to do a weekly lumbar puncture in all patients admitted with miliary tuberculosis, in order to detect an eventual onset of tuberculous meningitis. In this baby (see Table IV) the first lumbar puncture was performed at the end of her first stay at this Hospital, at which time there were no meningeal signs whatsoever, and the cerebro spinal fluid was sent to the Laboratory for a bacteriological control. Four more cerebro spinal fluids were sent at weekly intervals. The biochemical findings were normal in all five specimens and there was only a slight increase in the number of cells. Yet two out of the five cerebro spinal fluids (the second and the fifth week specimen) showed growth of Mycobacterium tuberculosis. The first isolated strain was sensitive to 1 gamma per ml. of streptomycin. As soon as the bacteriological finding was reported, 36 days after admission, the patient started to receive 15 milligrams per dose of intrathecal streptomycin, constantly given until the baby's death, 10½ months after admission. During the first course of treatment, which lasted almost seven weeks, tubercle bacilli were isolated 3 times: twice during the first week, once in the sixth week (see Table V). At the beginning of treatment the patient was gaining weight and doing well. Towards the end of the first course of intrathecal therapy her general condition looked stationary, with no remarkable changes. During the second course of intrathecal treatment a surprising number of positive cultures of Mycobacterium tuber-

TABLE IV

Mycobacterium tuberculosis isolated before Intrathecal Treatment from Cerebro Spinal Fluid of an infant who died.

Patient's Name and Age	June 12	June 16	June 29	July 6	July 10	Total No. of Positive Cultures
#22. D.B., 9 months old						
No.of C.S.F. Tested	1	1	1	1	1	
No.of Positive Cultures.	-	1	-	-	1	2
Gamma per ml. of streptomycin to which tubercle bacilli are sensitive.		1			1	

C.S.F. indicates Cerebro Spinal Fluid, - indicates No Growth.

TABLE V

Mycobacterium tuberculosis isolated during the 1st course of intrathecal treatment from Cerebro Spinal Fluid of an infant who died.

Patient's Name and Age	During 1st Course of Treatment							Total No. of Positive Cultures
	I week	II week	III week	IV week	V week	VI week	VII week	
#22. D.B., 9 months old								
No. of C.S.F. Tested	7	7	7	7	7	7	4	
No. of Positive Cultures.	2	-	-	-	-	1	-	3

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

culosis was obtained (see Table VI). This was an unusual finding. All patients, with few exceptions, had negative cultures of Mycobacterium tuberculosis from the cerebro spinal fluids taken after the first course of treatment. In this baby tubercle bacilli were isolated 17 times during the second course: twice in the 1st & 2nd weeks, four times in the 3rd & 4th weeks, four in the 5th & 6th weeks, four in the 7th & 8th weeks and three in the 9th week. The two strains isolated during the 1st & 2nd week were sensitive to 1 gamma per ml. of streptomycin, so were one of those isolated in the 3rd & 4th week, the 5th & 6th week and the 9th week. Parallel with these positive findings ran the baby's general condition which, at the end of the 2nd course of intrathecal therapy, were bad and the patient was two pounds below the admission weight. The third course of intrathecal therapy was started and again Mycobacterium tuberculosis was cultured eleven times from her cerebro spinal fluids (see Table VII). At the end of the 5th week of the third course of intrathecal therapy, P.P.D. administration was discontinued, but intrathecal streptomycin was still given at the dose of 15 milligrams every second day. Sensitivity tests were performed on some of the strains of Mycobacterium tuberculosis isolated during the third course of intrathecal treatment and the results were as follows: two strains of the 1st & 2nd weeks, one of the 3rd & 4th weeks, one of the 5th week, all were equally sensitive to 1 gamma per ml. of streptomycin and to 0.05 gamma per ml. of INH

TABLE VI

Mycobacterium tuberculosis isolated during the 2nd course of intrathecal treatment from Cerebro Spinal Fluid of an infant who died.

Patient's Name and Age	During IInd Course of Treatment					Total No. of Positive Cultures
	I & II week	III & IV week	V & VI week	VII & VIII week	IX week	
#22. D.B., 9 months old.						
No.of C.S.F. Tested	7	7	7	7	3	
No. of Positive Cultures.	2	4	4	4	3	17
Gamma per ml. of streptomycin to which tubercle bacilli are sensitive.	1 1	1	1		1	

C.S.F. indicates Cerebro Spinal Fluid.

TABLE VII

Mycobacterium tuberculosis isolated during the IIIrd course of intrathecal treatment from Cerebro Spinal Fluid of an infant who died.

Patient's Name and Age	During IIIrd Course of Treatment			Total No. of Positive Cultures.
	I & II week	III & IV week	V week	
#22. D.B., 9 months old.				
No. of C.S.F. Tested	7	7	4	
No. of Positive Cultures.	6	4	1	11
Gamma per ml. of streptomycin to which tubercle bacilli are sensitive.	1 1	1	1	
Gamma per ml. of INH to which tubercle bacilli are sensitive.	0.05	1	0.05	

C.S.F. indicates Cerebro Spinal Fluid, INH indicates Isonicotinic Acid Hydrazide.

N.B. After the end of the IIIrd course of treatment, intrathecal administration of P.P.D. was discontinued, while intrathecal streptomycin was continuously given at the dose of 15 milligrams every second day until the baby's death, four months later. Mycobacterium tuberculosis was isolated twice after P.P.D. was discontinued, 31 and 35 days later. These 2 last isolated colonies of tubercle bacilli were both sensitive to 1 gamma per ml. of streptomycin and to 0.05 gamma per ml. of INH.

(isonicotinic acid hydrazide) in the 1st & 2nd, and in the 5th week, but to 1 gamma per ml of INH in the 3rd & 4th week. (INH was started in the baby only during the third course of treatment). After intrathecal P.P.D. was discontinued, tubercle bacilli were not recovered from the cerebro spinal fluids for one month. At the end of the month two more positive cultures were obtained. The second culture of Mycobacterium tuberculosis, the last of the series, grew from a specimen taken 6 months and 11 days after the intrathecal therapy was started. This last isolated strain was also sensitive to 1 gamma per ml. of streptomycin and to 0.05 gamma per ml. of INH. It has to be pointed out that the sensitivity to streptomycin of the tubercle bacilli isolated during this long period of intrathecal treatment, remained unchanged. All the bacilli tested before, during treatment, and the last ones, all of them were equally sensitive to 1 gamma per ml. of streptomycin. Immediately after the last isolation of Mycobacterium tuberculosis a ventricular drain was placed in the brain, because of a marked hydrocephalus. The drain was kept for two months and 18 days, until the baby's death. During all this last period a considerable amount of cerebro spinal fluid (from 100 to 200 cc.) was sent to the laboratory every day. We were confronted with technical problems. The fluid, collected in a bottle during 24 hours, always contained air contaminants. In order to destroy them, the specimens had to be treated with alcalies and neutralized with acids, the amount of fluid was too voluminous, and we could not use the membrane filters. The

cultures of the sediment of the centrifuged fluid on Loewenstein slopes were always negative.

In this patient we could date the onset of tuberculous meningitis with great accuracy and treatment was started immediately. The persistance of Mycobacterium tuberculosis in the cerebro spinal fluid through the disease, cannot be correlated with an increased resistance to streptomycin. Mycobacterium tuberculosis isolated from this treated case was always equally sensitive to this drug. This unusual lasting positivity of the cerebro spinal fluid has to be ascribed partially to an insufficient dose of streptomycin.

Patient #23, ten months old, was admitted with tuberculous meningitis and primary pulmonary tuberculosis (see Table VIII). Fifteen milligrams of intrathecal streptomycin and daily lumbar punctures were started on admission. For the first ten days all cultures were negative, an uncommon finding in our series. Intrathecal P.P.D. was given on the 10th day and the first two positive cultures were obtained four days later. Ten more positive cultures were isolated during the next weeks until the end of the first course of therapy as it is shown in the table. The first strain of Mycobacterium tuberculosis at the end of the second week of treatment was sensitive to 1 gamma per ml. of streptomycin. one, in the 4th week, was sensitive to 0.1 gamma per ml., the two last ones, in the 6th week, were sensitive to 1 gamma per ml. Although the bacteriological findings were positive throughout the first course of intrathecal treat-

TABLE VIII

Mycobacterium tuberculosis isolated from Cerebro Spinal Fluid of patients discharged cured, who received 15 milligrams of intrathecal streptomycin per dose.

Patient's Name and Age	Before Treatment	During I Course of Treatment						Total No. of Positive Cultures.
		I week	II week	III week	IV week	V week	VI week	
#23. M.L., 10 months								
No. of C.S.F. Tested	1	7	7	7	7	7	3	
No. of Positive Cultures.	-	-	1	3	3	2	2	11
#24. R.B., 7 months								
No. of C.S.F. Tested	1	7	7	6	7	7	6	
No. of Positive Cultures.	-	4	3	-	2	7	2	18
#25. R.A., 5 months								
No. of C.S.F. Tested	1	7	7	7	7	6	4	
No. of Positive Cultures.	1	6	7	7	3	1	-	27

Mycobacterium tuberculosis was isolated twice during the 5th month of therapy, from the C.S.F. of this baby.

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

ment, this patient was making good clinical progress. She was discharged as cured and an electroencephalogram, taken on discharge, did not show brain damage.

Patient #24, an infant seven months old, was admitted with tuberculous meningitis (see Table VIII). There was no radiological, nor bacteriological evidence of pulmonary tuberculosis. Intrathecal streptomycin, 15 milligrams per day, was given on admission, and this same dose was continued for the duration of treatment. Tubercle bacilli were repeatedly isolated, 18 times, from his cerebro spinal fluids during the first six weeks of therapy. Sensitivity tests to streptomycin were performed on strains isolated during the 1st, 2nd and 5th week of treatment, and on the last strain obtained from a specimen during the 6th week of therapy. The results were as follows: tubercle bacilli sensitive to 1 gamma per ml. of streptomycin the 1st week, to 0.1 gamma per ml. the 2nd and 5th week, to 10 gamma per ml. the 6th week. There was a slight increased resistance to streptomycin in the last isolated strain. However, tubercle bacilli were not cultured any longer after the 6th week until the end of treatment. The patient has been discharged home because the tuberculous meningitis was considered cured. In spite of the cure of the meningeal inflammatory process, an electroencephalogram, taken 20 days after the course of therapy was completed, showed the following changes: "This record is very abnormal. The shifting epilectic foci (As compared with the previous record taken nine months ago) still indicates, in addition the abnormal

background activity, a severe involvement of brain structure. There is evidence in this examination of potentially epilectic activity in the left fronto central and temporal regions". The patient was discharged home having still frequent spastic convulsions.

Patient #25, 5 month old, was admitted because of tuberculous meningitis and pulmonary tuberculosis (see Table VIII). Tubercle bacilli have been cultured 27 times from her cerebro spinal fluids during the first five months of intrathecal therapy. Mycobacterium tuberculosis grew from 25 cerebro spinal fluids, out of 37 tested, during the first 5 weeks of the first course of therapy. For all this period the patient was given 15 milligrams per day of intrathecal streptomycin. It has to be noted that the first strain of tubercle bacilli, isolated from the first cerebro spinal fluid taken on admission, before intrathecal treatment was started, was sensitive only to 10 gamma per ml. of streptomycin and isonicotinic acid hydrazide. (This patient was given INH daily: 50 milligrams per day during the first course of treatment, 25 milligrams for the remaining 160 days). Another strain, tested 22 days later, was sensitive to 0.1 gamma per ml. of streptomycin and to 1 gamma per ml. of INH. As soon as the relatively resistant strain was reported, on the 6th week of the first course of treatment, the patient started to receive 50 milligrams of intrathecal streptomycin every second day for a period of a month. Thereafter the doses were reduced to 25 milligrams every second day for 48 days. At the end of the

48 days, intrathecal streptomycin was given again at the doses of 15 milligrams. It has to be pointed out that for all the period during which the patient was receiving 50 milligrams of intrathecal streptomycin for 30 days, and 25 milligrams for 48 days, tubercle bacilli did not grow from her cerebro spinal fluids. But Mycobacterium tuberculosis grew from two specimens taken 6 and 7 days after the patient was put back to the dosage of 15 milligrams of intrathecal streptomycin. Both the last two positive cultures were sensitive to 0.1 gamma per ml. of streptomycin and to 0.05 gamma per ml. of isonicotinic acid hydrazide. After these two positive findings the patient has been put back to 25 milligrams of intrathecal streptomycin every second day until the end of therapy.

It has to be noted that the baby was doing very poorly for all the first 40 days of treatment, during which she was kept at the dosage of 15 milligrams a day of intrathecal streptomycin. Not only was she not gaining weight, but she was loosing it and looking very miserable. On admission her weight was 13 pounds, 7½ ounces; 37 days later it was 12 pounds, 15½ ounces. The first slight clinical improvement was noted twelve days after the first administration of 50 milligrams of intrathecal streptomycin. One month after the start of the increased therapy she weighed 14 pounds, 9 ounces and constant progress has been noted since then. During the period of reduced dosage of intrathecal streptomycin, 15 milligrams every two days for 32 days, her appearance remained stationary and her general condition did

not get worse. Biochemical tests were not performed in the cerebro spinal fluid from which the first of the last 2 cultures of Mycobacterium tuberculosis was obtained. The second of these spinal fluids had increased proteins, but this finding was due to the considerable amount of blood present in the specimen. Subsequent cerebro spinal fluids, until treatment was completed, did not show pathological modifications of biochemical tests, and bacteriological findings were always negative. However, the reappearance of tubercle bacilli after two months of therapy and the lack of clinical improvement, both in connection with the reduced doses of 15 milligrams of intrathecal streptomycin, were another indication that such dosage was inadequate. The patient was discharged home still spastic, but cured of her tuberculous meningitis. An electroencephalogram, taken 12 days after treatment was completed, shows what follows: "The E.E.G. confirms the impression of the previous one (taken four months ago), that there is diffuse brain damage. There is at this time an epilectogenic focus in the right central region near electrode C4".

In spite of the fact that 1 out of 4 patients, who were treated with 15 milligrams per dose of intrathecal streptomycin, recovered well from tuberculous meningitis, there was much bacteriological and clinical evidence for the other 3 infants, that this dosage was insufficient. Attempts of intrathecal therapy in infants with 15 milligrams per doses have been abandoned at the Alexandra Hospital.

(B) Patients who Received 25 Milligrams Per Dose of Intrathecal Streptomycin. Twenty-eight patients were given 25 milligrams per dose of intrathecal streptomycin. Three of them died; twenty are cured; five are still under treatment.

(a) Patients who Died (see Table IX). Patient #26, 5 years old, had positive cultures until the end of the first course of treatment. Tubercle bacilli were not isolated during the four weeks of the second course: the cerebro spinal fluids were all partially clotted and filtration was incomplete. It has to be pointed out that Mycobacterium tuberculosis, isolated during the first week of treatment, was sensitive to 1 gamma per ml. of streptomycin, while the last isolated strain was resistant to 100 gamma per ml. This is the only strain in our series, which became resistant to streptomycin.

Patient #27, eleven years old, had extensive bilateral cavitary lesions in the lungs and diabetes mellitus as well. Tubercle bacilli were isolated repeatedly until his death. 17 out of 24 specimens showed growth of Mycobacterium tuberculosis, which were sensitive to 0.1 gamma per ml. of streptomycin.

Patient #28, 2 years old, died shortly after admission and 5 out of 6 cerebro spinal fluids, had growth of tubercle bacilli.

TABLE IX

Cerebro Spinal Fluid cultured only on membrane filter, from patients who died and who received 25 milligrams of intrathecal streptomycin per dose.

Patient's Name and Age	Before Treatment	During I Course of Treatment						I to V Week of Second Course of Treatment	Total No. of Positive Cultures
		I week	II week	III week	IV week	V week	VI week		
#26. Ro.L., 5 years.									
No.of C.S.F.Tested	1	7	7	7	7	7	7	* 34	
No.of Positive Cultures	-	1	-	-	1	1	1	- DIED	4
#27. B.C., 11 years.									
No.of C.S.F.Tested	3	7	7	7	DIED				
No.of Positive Cultures	3	7	5	2					17
#28. A.N., 2 years.									
No.of C.S.F.Tested	2	4							
No.of Positive Cultures	1	4	DIED						5

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

* All the specimens received during the second course of treatment were partially clotted and filtration was incomplete.

(b) Patients Discharged As Cured. Twenty patients were discharged as cured. Eleven of them received only the treatment previously described (see Table X); nine of them were given in addition isonicotinic acid hydrazide (INH) daily, 15 milligrams per kg. during the first course of treatment, 10 milligrams per kg. for the next days, until the end of treatment (see Table XI).

Bacteriological diagnosis was established in 18 of the 20 patients that were discharged as cured. From one of the two patients on whom tuberculous meningitis was not bacteriologically proved, an unindentifiable and fast organism was isolated. The second patient was treated elsewhere, before admission to the Alexandra Hospital, with intrathecal streptomycin and P.P.D. for six months, without bacteriological evidence at any time of tuberculous meningitis.

In nine patients we obtained only from one to five cultures of Mycobacterium tuberculosis. Two of them were seriously ill, while the other seven only mildly, and in three of these the diagnosis of tuberculous meningitis was in doubt until the bacilli were isolated.

In six other patients, 7 to 15 positive cultures were obtained. From three children tubercle bacilli were cultured 21, 21 and 23 times during treatment.

(c) Patients Still Under Treatment. All the five children, still under treatment, were proved to be tuberculous meningitis by repeated isolation, and from one 18

TABLE X

Eleven patients discharged cured, who received 25 milligrams of intrathecal streptomycin per dose, but no isonicotinic acid hydrazide.

Patient's Name and Age	Before Treatment	During I Course of Treatment						Total No. of Positive Cultures
		I week	II week	III week	IV week	V week	VI week	
#29. L.C., 4 years								
No. of C.S.F. Tested	2	7	7	7	7	7	7	
No. of Positive Cultures.	2	-	-	-	-	-	-	2
#30. H.J., 5 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	1	-	-	-	-	-	-	1
#31. L.P., 12 years.								
No. of C.S.F. Tested	4	7	7	7	7	7	7	
No. of Positive Cultures.	1	1	-	-	-	-	-	2
#32. P.S., 5 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	-	1	2	-	-	-	-	3
#33. R.L., 3 years.								
No. of C.S.F. Tested	3	7	7	7	7	7	7	
No. of Positive Cultures.	3	4	1	-	-	-	-	8
#34. Y.D., 12 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	1	6	1	1	-	-	-	9

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

TABLE X (Continued)

Eleven patients discharged cured, who received 25 milligrams of intrathecal streptomycin per dose, but no isonicotinic acid hydrazide.

Patient's Name and Age	Before Treatment	During I Course of Treatment						During II Course of Treatment		Total No. of Positive Cultures
		I week	II week	III week	IV week	V week	VI week	I week	II week	
#35. B.R., 5 years.										
No.of C.S.F.Tested	2	7	7	7	7	7	4	3	4	
No.of Positive Cultures	2	5	4	5	3	1	1	-	-	21
#36. J.B., 6 years.										
No.of C.S.F.Tested	1	7	7	7	7	7	7	6	7	
No.of Positive Cultures	1	4	-	2	2	4	-	1	1	15
#37. D.A., 3 years.										
No.of C.S.F.Tested	1	7	6	7	7	7	4	3	4	
No.of Positive Cultures	1	7	1	5	2	4	-	-	1	21
#38. Y.P., 11 years.	An unidentifiable organism, partially acid fast, was isolated. Tubercle bacilli were never grown.									
#39. A.S., 4 years.	Tubercle bacilli were never isolated. This patient was treated elsewhere with intrathecal streptomycin & P.P.D. for a period of 6 months before admission to the Alexandra Hospital.									

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

TABLE XI

Nine patients discharged cured, who received 25 milligrams of intrathecal streptomycin per dose and isonicotinic acid hydrazide.

Patient's Name and Age	Before Treatment	During I Course of Treatment						Total No. of Positive Cultures
		I week	II week	III week	IV week	V week	VI week	
#40. G.W., 14 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	-	1	-	-	-	-	-	1
#41. J.E., 7 years.								
No. of C.S.F. Tested	2	7	7	7	7	7	7	
No. of Positive Cultures.	2	5	-	-	-	-	-	7
#42. G.G., 2 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	1	2	-	-	-	-	-	3
#43. W.P., 4 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	-	2	2	-	-	-	-	4
#44. A.W., 5 years.								
No. of C.S.F. Tested	None	7	7	7	7	7	7	
No. of Positive Cultures.		1	2	-	-	-	-	3

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

TABLE XI (Continued)

Nine patients discharged cured, who received 25 milligrams of intrathecal streptomycin per dose and isonicotinic acid hydrazide.

Patient's Name and Age	Before Treatment	During I Course of Treatment						Total No. of Positive Cultures
		I week	II week	III week	IV week	V week	VI week	
#45. L.F., 4 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	7	
No. of Positive Cultures.	-	4	1	-	-	-	-	5
#46. E.S., 11 years.								
No. of C.S.F. Tested	3	7	7	7	7	7	7	
No. of Positive Cultures.	-	4	4	1	-	-	-	9
#47. J.V., 3 years								
No. of C.S.F. Tested	1	7	7	7	6	6	4	
No. of Positive Cultures.	-	-	-	1	6	2	-	9
#48. J.M., 8 years.								
No. of C.S.F. Tested	1	7	7	7	7	7	4	
No. of Positive Cultures.	1	7	6	5	3	-	1	23

- indicates No Growth, C.S.F. indicates Cerebro Spinal Fluid.

cultures were obtained during the whole six weeks of treatment. Final results cannot be given as yet, because the full series of tests have not been completed.

SENSITIVITY TO STREPTOMYCIN AND ISONICOTINIC ACID HYDRAZIDE

Sensitivity to streptomycin was tested in all patients on the first strain of Mycobacterium tuberculosis isolated from the cerebro spinal fluids, as well as on the intermediate and on the last one, according to the number of positive cultures obtained. Sensitivity to isonicotinic acid hydrazide was performed only on the strains from the patients who received it.

Only in one patient (#26), who died, Mycobacterium tuberculosis became resistant to streptomycin. The last isolated strain, in the sixth week of treatment, was not sensitive to 100 gamma per ml. of streptomycin, while the first one, during the first week of treatment, was sensitive to 1 gamma per ml.

Four other patients had a strain which was sensitive to 10 gamma per ml. of streptomycin. In two patients such strain was an intermediate, while the other strains, tested previously and after, were all very sensitive to streptomycin, to concentrations varying from 0.1 to 1 gamma per ml. In the

third patient the strain which showed an increased resistance to streptomycin was the last isolated, in the sixth week of treatment, while previous strains, in the first, second and fifth week of treatment, were sensitive to 1, 0.1, 0.1 gamma per ml. respectively. In the fourth patient (#25) an unusual finding was obtained. The first strain of Mycobacterium tuberculosis, cultured from a cerebro spinal fluid before intrathecal treatment was started, was sensitive to 10 gamma per ml. of streptomycin and isonicotinic acid hydrazide. Tubercle bacilli, isolated from the same patient in the third week of treatment, were sensitive to 0.1 gamma per ml. of streptomycin and to 1 gamma per ml. of INH. The two last strains, in the fifth month of therapy, both were sensitive to 0.1 gamma per ml. of streptomycin and to 0.05 gamma per ml. of isonicotinic acid hydrazide.

All the other strains of Mycobacterium tuberculosis, from the remaining patients, were all sensitive to concentrations of streptomycin, varying from 0.1 to 1 gamma per ml. The strains tested for isonicotinic acid hydrazide all showed to be very sensitive to this drug.

CORRELATION BETWEEN THE INTRATHECAL P.P.D. INJECTION AND THE BACTERIOLOGICAL FINDINGS

Tubercle bacilli were isolated before intrathecal P.P.D. treatment from all our patients, but four, whose cerebro spinal fluids had growth of Mycobacterium tuberculosis only after P.P.D. administration.

In patient #23 (see Table VIII) intrathecal streptomycin and daily lumbar punctures were started on admission. All the cultures were negative for the first ten days. Intrathecal P.P.D. was given on the 10th day and the first positive culture was obtained four days later. Ten more positive cultures were found in the next twenty-five days.

Patient #26 (see Table IX) was given intrathecal P.P.D. on admission. The cerebro spinal fluid before P.P.D. injection was negative, while the specimen taken 24 hours later had growth of Mycobacterium tuberculosis.

In patient #32 (see Table X) intrathecal P.P.D. was started on admission, but cultures were negative prior to and for the first six days of treatment, after which she was positive on three occasions.

Patient #47 (see Table XI) was given intrathecal P.P.D. on the eighth day from admission. The first administration of 2.5 gamma was followed by a reaction. Tubercle bacilli were not isolated. A second administration of 2.5 gamma was given, reaction did not take place, no tubercle bacilli were isolated from the cerebro spinal fluids. Two days later 5 gamma of P.P.D. were injected for three times at the interval

of four days one from the other. Reaction took place after every injection. Tubercle bacilli did not grow after the first two injections. Immediately after the reaction, which followed the third administration of P.P.D., a cerebro spinal fluid had growth of Mycobacterium tuberculosis. Eight more specimens showed tubercle bacilli during the next 13 days.

Correlation during treatment between positivity of cultures and reaction to P.P.D. injection, is difficult. Reactions following P.P.D. introduction were present more often than not.

An interesting observation was made on Patient #24 (see Table VIII) while intrathecal P.P.D. was given. Before P.P.D. was started, two out of four specimens had growth of Mycobacterium tuberculosis. On the first culture 3 colonies were growing, on the second one 2 colonies. A dose of 1.25 gamma of P.P.D. was given, reaction did not occur. The cerebro spinal fluid taken the following day showed no growth of tubercle bacilli, and that one, taken two days later, had growth of two colonies of Mycobacterium tuberculosis. P.P.D. was doubled, 2.5 gamma, two days after the first administration; reaction took place. The cerebro spinal fluid taken the following day showed a profuse growth of Mycobacterium tuberculosis, 64 colonies.

BACTERIOLOGICAL CONTROL OF THE CLINICALLY RECOVERED PATIENTS

32 children, discharged as cured, came back to the Hospital for a control and all their cerebro spinal fluids had negative cultures. One of them was readmitted because he was thought to have a relapse of tuberculous meningitis and he was given a complete course of intrathecal therapy. Tubercle bacilli were never isolated from the 102 spinal fluids, cultured during his stay at the hospital.

CONCLUSIONS

Why not centrifuged specimen?

1. Ordinary method of culturing cerebro spinal fluid on Loewenstein slopes, inoculating each of 4 tubes with one cc. from every specimen as received, proved not to be satisfactory for the isolation of tubercle bacilli from the cerebro spinal fluids of tuberculous meningitis patients. Only 12 out of 16 patients were bacteriologically proved on culture in this way.

2. Collection on a filter membrane has been introduced as a routine culture method for the isolation of Mycobacterium tuberculosis from the cerebro spinal fluid.

3. 35 patients out of 37 were bacteriologically proved

to be tuberculous since the filtration technique was used. Of the two failures, one had isolated an unidentifiable acid fast organism; the second was treated elsewhere, before admission, with intrathecal streptomycin and P.P.D. for six months, without bacteriological evidence of tuberculous meningitis at any time.

4. Membrane filter technique was shown to be effective in detecting tubercle bacilli when the meningeal reaction is minimal and the bacilli presumably few in number. In one patient we could date the onset of tuberculous meningitis with great accuracy, when meningeal signs were absent and the biochemical findings in the cerebro spinal fluid still normal.

5. From the 35 proved tuberculous meningitis patients, on whose cerebro spinal fluids membrane filters were used, Mycobacterium tuberculosis could be isolated for different lengths of time during treatment. Mycobacterium tuberculosis was not usually cultured beyond the 4th week of treatment from the cerebro spinal fluid of patients who had evident clinical improvement. Two exceptional cases remained seriously ill for 7 and 10 weeks, but had positive cultures only during the first and the two first weeks of treatment respectively, although their cerebro spinal fluids were cultured every day during all this period. In the seven fatal cases cultures were obtained almost throughout the period of illness.

These findings by filtration technique contrast strongly with the results of direct culture on Loewenstein slopes, on which no isolation was possible during treatment with streptomycin and with only an average of less than two positive cultures per case. The medium is not implicated because the membranes were planted on the surface of Loewenstein medium. It should be pointed out that the difference in results by the two methods remains, although the intrathecal streptomycin was reduced from 50 milligrams to 25 per dose, according to the child's age, prior to the introduction of the filtration technique. However, the improved method of isolation may indicate need for revision of the course of treatment.

6. Sensitivity of the isolated strains to streptomycin was tested in all patients and sensitivity to isonicotinic acid hydrazide only in those who received it. In only one patient, who died, the last isolated culture of Mycobacterium tuberculosis, in the six week of treatment, showed an increased resistance from 1 gamma to 100 gamma per ml. of streptomycin. In all patients all the strains, tested before and during treatment, remained sensitive from 0.1 gamma to 1 gamma per ml. of streptomycin. However, in four patients one of the tested strains was sensitive only to 10 gamma per ml. of streptomycin. The strains tested for isonicotinic acid hydrazide were sensitive from 0.05 to 1 gamma per ml., with only one exception in a patient who had a strain sensitive to 10 gamma per ml. of INH and strepto-

mycin. It is difficult to establish the clinical significance of these fluctuations in the degree of sensitivity of tubercle bacilli. In four patients relatively resistant strains were present, but the cerebro spinal fluids still contained sensitive organisms. The four patients have all responded to further treatment with the tested drugs.

7. Correlation between the reaction following P.P.D. injection and the incidence of positive cultures, is difficult. Tubercle bacilli were not isolated before intrathecal P.P.D. was started in four of our patients. In a fifth there was evident liberation of tubercle bacilli after the tuberculin reaction took place. These observations are significant because they indicate the effect of P.P.D. and suggest its importance in the treatment of tuberculous meningitis.

8. Bacteriological control of the clinically recovered patients showed constantly negative cultures so far.

SUMMARY OF RESULTS

The filtration technique as a routine culture method for the isolation of Mycobacterium tuberculosis from the cerebro spinal fluid of tuberculous meningitis patients shows great improvement over methods used previously.

The technique is shown to be effective in detecting tubercle bacilli when the meningeal reaction is minimal and the bacilli presumably few in number.

Its use, during combined intrathecal treatment with streptomycin and Purified Protein Derivative of tuberculin, shows that the organisms persist for an unexpectedly long time in the cerebro spinal fluid. Direct culture of cerebro spinal fluid on Loewenstein medium gave less than two positive specimens per patient and no positive cultures later than the second week of treatment. Filtration gave more profuse growth and persistance of positive cultures during treatment for as long as 28 weeks in some cases.

Tubercle bacilli did not show increased resistance to streptomycin, with the exception of one strain.

In some instances, focal reaction to P.P.D. seems to liberate tubercle bacilli from the lesions into the fluid.

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