STUDIES OF THE CEREBROSPINAL FLUID CIRCULATION IN TUBERCULOUS MENINGITIS IN CHILDREN

PART I. THE USE OF PENICILLIN AS A TRACER SUBSTANCE

BY

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It has been conclusively proved that the cerebrospinal* route for the administration of streptomycin is essential in the treatment of tuberculous meningitis (Medical Research Council, 1948; Rubie and Mohun, 1949). Only a few workers consider it unnecessary (Levinson, 1949; Mouriquand, 1948). There is, however, less general agreement about the optimum site of cerebrospinal administration of streptomycin. The lumbar, ventricular, and cisternal routes are most commonly used, but streptomycin has also been injected through indwelling polyethylene catheters direct into the interpeduncular space (Cairns, 1949; Smith, Vollum, and Cairns, 1948) or into the pontine cistern (Komrower, 1950).

Early in the experimental stage of streptomycin treatment of tuberculous meningitis it became obvious that obstruction within the cerebrospinal pathways was frequent and that this factor must influence the choice of route. The sites of obstruction are the same as those in other forms of meningitis, namely the Sylvian aqueduct, the foramina of Magendie and Luschka in the roof of the fourth ventricle, and especially at the tentorial opening and within the spinal theca (Cairns, 1949). There are various views regarding the aetiology of these blocks. Tuberculous granulation tissue (M.R.C., 1948); fibrinous exudate which may become fibrous later (Cairns, 1949; Cathie, 1949; Smith *et al.*, 1948; Daniel, 1949); reaction to 'bloody tap' (Cairns and Taylor, 1949); the irritant effect of streptomycin (Cathala and Bastin, 1948; Winter, 1950); and herniation of the brain into the foramen magnum or tentorial opening (Cairns, 1949; Smith *et al.*, 1948) have all been blamed. These may be responsible singly or in combination. Early recognition of these blocks is necessary because they have an important therapeutic and prognostic significance. Such early

recognition can be achieved by the systematic study of the circulation of the cerebrospinal fluid.

Dandy and Blackfan (1914) and Dandy (1919) have shown in a series of classical experiments that the cerebrospinal fluid is formed by the choroidal plexuses of all four ventricles. The fluid passes through the foramen of Monro into the third ventricle, the narrow Sylvian aqueduct and the fourth ventricle and then emerges from the brain through the medial and lateral foramina of the roof of the fourth ventricle. Part of the fluid travels down and then up inside the spinal subarachnoid space to join the rest at the cisterna magna at the cerebello-medullary angle. The fluid here divides again. Some of it travels forward via a system of basal cisterns, the remainder passing upward over the cerebellum, then under the tentorium to join the rest and traverses the final narrowing at the tentorial opening, where the cisterna ambiens encircles the brain stem. The fluid then rises above the tentorium into the subarachnoid space over the surface of the brain. According to Dandy and Blackfan (1913) and Dandy (1929) most of it is absorbed here into the capillaries of the pia-arachnoid, and not into the great sinuses via the arachnoid villi, as was originally held by Key and Retzius (1875-76). Weed's (1914, 1922, and 1935) experiments and observations, however, strongly supported the view of the latter workers which is largely accepted at present. Under pathological conditions the choroid plexuses (Foley, 1923; Forbes, Fremont-Smith, and Wolff, 1928) and possibly the ependymal lining of the ventricles may act as absorbing surfaces (Nañagas, 1921; Wislocki and Putnam, 1921). A relatively insignificant proportion of fluid is absorbed in the spinal subarachnoid space and in the posterior fossa of the brain (Dandy and Blackfan, 1914).

In the absence of a block the cerebrospinal fluid obtained by lumbar, cisternal or ventricular puncture is of very similar composition, although the protein

* 'Cerebrospinal' is used as an inclusive term for the intrathecal (lumbar and cisternal) and intraventricular route.

concentration in the lumbar sac is usually slightly above that found at a higher level (Greenfield and Carmichael, 1925; Merritt and Fremont-Smith, 1937a). Constriction of the jugular veins during lumbar puncture usually results in a considerable and rapid rise of pressure, followed by an equally rapid fall on release of the constriction (Queckenstedt, 1916).

The usual signs of spinal block are a rising protein content of the cerebrospinal fluid, a rapid fall in pressure, and an absence of rise of pressure on jugular compression. The presence of a block is more certain if there is a gross difference between the protein content of the lumbar and the cisternal fluid (Ayer, 1922), although it is known that when there is an increase in the C.S.F. protein, that increase is greatest in the lumbar fluid (Merritt and Fremont-Smith, 1937b). These are unfortunately late signs of a spinal block in tuberculous meningitis, and yet they are not infallible. Very high protein, low pressure, and the slow dripping of fluid singly or in combination may occur without a true block (Cairns and Taylor, 1949). The signs cannot be relied on at all if the block occurs above the cisterna magna, as for example at the tentorial opening or the Sylvian aqueduct. In such cases the rate of flow of the cerebrospinal fluid may be normal or even accelerated and the protein content is not above the usual level in tuberculous meningitis.

Earlier and more accurate diagnosis of a spinal block or of blocks at a higher level can be accomplished by injecting dyes (Dandy, 1918) or antibiotics (Cairns, Duthie, Lewin, and Smith, 1944; Smith and Daniel, 1947) into the cerebrospinal pathways at one point and detecting their presence after a suitable interval at a different point. Lipiodol and allied compounds have been used extensively in neurosurgery for the detection of spinal blocks since the description of the method by Sicard and Forestier (1921 and 1926), but is contraindicated because of the local irritation they cause and the difficulty of removing them after injection. The detection of blocks by air studies will be reviewed in detail in Part II.

Milani-Comparetti, Zoli, and Pasquinucci (1948 and 1949) compared the streptomycin content of the lumbar and cisternal fluid eight hours after lumbar injection in a large series of cases of tuberculous meningitis. They found that normally the same values were obtained and the 'diffusion index' of cisternal lumbar streptomycin content was 1. If the value of this index was below 1 partial block was suspected and absence of streptomycin in the cisternal fluid was accepted as proof of complete spinal block. In such cases they considered that cisternal administration of streptomycin was essential.

The Oxford workers (Smith and Daniel, 1947) did not find the use of dyes very satisfactory in cases of meningitis and suggested the use of penicillin as a tracer substance in streptomycin-treated tuberculous meningitis. Its advantages are that it is non-toxic, non-irritant in therapeutic doses, easy to assay quantitatively, and can be used repeatedly. It is probably safe to assume that where penicillin can penetrate, streptomycin will do likewise. The method adopted at Oxford was to inject penicillin into one of the lateral ventricles and to detect its presence in the lumbar cerebrospinal fluid, or vice versa, two to six hours later. The disadvantages of this method are that the concentrations of penicillin obtained are usually quite low (Smith, personal communication), presumably due to absorption of penicillin into the general circulation; that two separate operations at different times are required; that if a block is detected its exact location is not identified; and finally, that cranial burrholes are necessary when the suture lines are closed.

Present Study

Method. In this study the Oxford technique was adopted with the following modifications. The penicillin was injected by the lumbar, cisternal or ventricular route, but the lumbar route was preferred because it is the commonest and simplest route for intrathecal streptomycin administration. Cerebrospinal fluid was obtained before penicillin injection and was used as a blank control. Penicillin, 15,000 units, dissolved in 5 ml. of normal saline was then injected to replace an equal volume of cerebrospinal fluid. If the injection was given by the lumbar route, then either cisternal or ventricular fluid was obtained within five to ten minutes of the injection and its penicillin content assayed. Only 1 to 2 ml. of fluid were removed at this time, so that factors of negative pressure and hastened circulation should not tend to increase the penicillin content of the test fluid and thereby create false experimental conditions. If more fluid were removed for any other reason, this was collected in a separate container. The dose of penicillin and the time factor were kept constant whichever route was used for the injection. The cisternal route was frequently used, because it is nearest to the site of the maximal inflammation at the base of the brain and because it enabled a block to be localized to the spinal canal or above it. The examinations were performed 24 hours after the last intrathecal streptomycin injection and the usual dose of streptomycin was injected intrathecally after the last specimen was removed.

The total cerebrospinal fluid volume was arbitrarily assumed to be 150 ml. in each case. Obviously some deviation from this figure in both directions must have occurred in many cases but probably not to a significant degree, bearing in mind the frequent dilatation of the ventricles in the smaller children. Theoretically the dose of 15,000 units of penicillin should therefore give a concentration of 100 units/ml. if complete and rapid

mixing takes place. As the second specimen of cerebrospinal fluid was removed within 10 minutes of the original injection, the factors of absorption into the blood stream and re-secretion into the cerebrospinal fluid can be ignored. This was verified in the earlier cases of the series, where no bacteriostatic penicillin level could be detected in the blood taken 10 minutes after the intrathecal injection. The bacteriological technique employed is described in the Appendix.

The protein content of the cerebrospinal fluid specimens obtained for the penicillin assays was also determined. These findings were correlated with those of the penicillin assays, and the latter were used as controls on the accuracy of protein levels and the lumbar/cisternal protein ratio as an index of spinal block.

Material. Twenty-seven children were selected for this method of investigation between January, 1949, and August, 1950. They were between 5 months and 12 years of age and all suffered from bacteriologically confirmed tuberculous meningitis. They were chosen from a much larger group of children treated with streptomycin for tuberculous meningitis, either because the presence of a block was suspected on clinical grounds, or else because it was desired to confirm the disappearance of a previously existing block. The examinations were performed up to seven times in an individual child, and altogether 55 penicillin assays have been performed. The sites investigated were between the lumbar theca and the cisterna magna in 38, between the lumbar theca and one or both lateral ventricles in 11, between the cisterna magna and the lateral ventricles in five, and between the two lateral ventricles in one (Table 1). No harmful effects were noted in any case.

Objective. There was an immediate and remote objective for these investigations. The immediate object was to establish the state of the cerebrospinal

fluid circulation in the individual case, and, if a block were detected, to take appropriate therapeutic steps. The remote objective was to discover how quickly penicillin (and thus streptomycin) reached the various parts of the system, what concentration could be obtained at the various points and thus to determine the optimum site of streptomycin injection not only in the individual case, but generally in all cases of tuberculous meningitis.

Results

It was soon realized that the exact site of blocks within the cranial cavity could be determined more accurately by air studies. The main part of the present investigation was therefore concerned with the detection of spinal blocks, but examinations were also carried out to detect blocks above that level, occurring either at the foramina in the roof of the fourth ventricle or at the Sylvian aqueduct. Penicillin assay is unsuitable for the detection of blocks at the tentorial opening because of the difficulty and often impossibility of obtaining fluid from the subarachnoid space over the surface of the brain. A tentorial block will not prevent penicillin from entering the ventricles from the lumbar theca or vice versa. This was proved several times by air encephalograms (Lorber, in the press).

In the absence of blocks no difference was found in the rate of dispersal of penicillin wherever it was injected. Penicillin could be detected in the ventricular or cisternal fluid after lumbar injection as quickly as the other way round, and apparently complete mixing took place within 10 minutes, both with, and against, the flow of the fluid. This observation strengthens the suggestion that there is not only an outward flow from the ventricles but that an active circulation of the fluid exists.

Spinal block was suspected in 20 children. Altogether 38 penicillin assays were performed on them, using lumbar and cisternal punctures. Spinal block was proved in 12 of these children on 18 occasions. In addition spinal block was regarded as proved in a further case. This 5-months-old infant was admitted following several convulsions. Lumbar puncture yielded scanty xanthochromic fluid under low pressure and with a protein content of 1,500 mg. %. Her fontanelle was bulging and was not influenced by removal of all available lumbar fluid. The protein content of the ventricular fluid was 50 mg. %. Penicillin assay was attempted next day and at a later date, but this could only be performed between the lateral ventricle and the cisterna magna, as lumbar fluid could not be obtained on any subsequent occasion. This child was the only one in which a spinal block was present before treatment. In all the others the block

TABLE 1
SITES INVESTIGATED BY PENICILLIN ASSAY AND RESULTS

	Number of Assays	Blocks Found	No Block
Lumbar theca to cisterna magna (or reverse) ..	38	18	20
Lumbar theca to lateral ventricles (or reverse) ..	11	4	7
Cisterna magna to lateral ventricles (or reverse) ..	5	1	4
Left to right lateral ven- tricle	1	0	1
Total	55	23	32

developed any time from within a few days to over a year after the beginning of treatment.

In one infant, the exact site of the spinal block was accurately localized to the cauda equina by high and low lumbar punctures.

The protein content of the lumbar and the cisternal cerebrospinal fluid was estimated on the specimens removed for the penicillin assay on 36 occasions. The penicillin assay indicated spinal block in half of them. The range of the protein content of the lumbar fluid was 65-1,500 mg. % in cases of block, and 20-1,200 mg. % without block, and the average was 340 and 280 respectively. There was a considerable overlap in the figures for the two sets of cases, although both the individual and average highest figures were obtained where a spinal block existed.

The ratio between the lumbar and cisternal protein content was also calculated. Where a block was present, the minimum ratio was 1.5:1.0, the maximum 30.0:1.0, and the average 3.8:1.0. In the absence of a block, the cisternal protein was slightly higher than the lumbar in one instance and the same in two. In the other 15 the lumbar/cisternal ratio varied from 1.1:1.0 to 7.2:1.0, and the average of all 18 estimations was 2.3:1.0. There was appreciable overlap in these ratios, too, and in seven of the 18 instances with a block the ratio was less than the average of 2.3:1.0 for the cases without blocks. Conversely, in three of the 18 instances without a block, the ratio was above 3.8:1.0, the average for those with blocks (Table 2). There is little doubt, however, that these findings apply only to cases where spinal block was suspected on clinical grounds, as only these children were investigated.

In eight children the clinical suspicion of a spinal block was disproved by penicillin assay. In one instance free spread of penicillin was observed from the lumbar to the cisternal area, in spite of a lumbar

protein value of 1,200 mg. % and a lumbar: cisternal protein ratio of 3.4:1.

In 10 children investigations for suspected block were carried out by penicillin assay on 16 occasions between the lumbar or cisternal area and the lateral ventricles or in the reverse direction. A block was found five times and these were found by encephalography to be at the Sylvian aqueduct or at the foramina in the roof of the fourth ventricle.

In the 12 fatal cases where necropsy was performed, the presence and location or absence of spinal or other blocks was found to correspond to the findings of the penicillin assay during life. Seven children had a spinal block at the time of death, but in only two could death be reasonably attributed directly to the spinal block. In both cases this developed in the first three weeks of treatment, was of rapid onset, and its recognition was too late. In the five other fatal cases a tentorial block leading to hydrocephalus was also present and this was considered to be the cause of death.

Spinal blocks were not necessarily permanent. In one fatal case blocks were proved on two occasions, but with normal spread of penicillin between these times. Six of the 13 children with spinal blocks are alive seven to 26 months after the beginning of treatment. In each of these it is assumed that the block no longer exists. In three of them, subsequent penicillin assays showed free circulation, in two others air freely entered the ventricles after lumbar injection at a later date, and in the sixth the lumbar cerebrospinal fluid returned to normal and stayed normal for over a year. The disappearance of these blocks was spontaneous in every case.

Discussion

The results of this investigation indicate that in the absence of a block there is no merit in administering streptomycin by the cisternal or ventricular route in preference to the lumbar one. Penicillin,

TABLE 2

CORRELATION OF THE PROTEIN CONTENT OF THE LUMBAR FLUID AND OF THE LUMBAR/CISTERNAL PROTEIN RATIO WITH SPINAL BLOCK AS INDICATED BY PENICILLIN ASSAY

	Protein Content of Lumbar C.S.F. (mg. %)		Lumbar C.S.F. Protein Ratio Cisternal	
	Range	Average	Range	Average
Spinal block (18 cases) ..	65-1,500	340	1.5-30.0 1	3.8 1
No spinal block (18 cases) ..	20-1,200	280	0.9-7.2 1	2.3 1

and therefore streptomycin, will freely reach all areas within a few minutes of lumbar injection and the concentration will be the same in the cisterna magna and in the ventricles. Penicillin rapidly diffuses from the site of injection if given by cisternal puncture.

There is a widespread belief that tentorial block, or the hydrocephalus due to it, necessitates ventricular or cisternal injections. Careful consideration of the anatomy of the cerebrospinal pathways will make it obvious that this is not the case, because there is still free communication between the lateral ventricles and the lumbar theca. This was fully borne out by the experiments described. There was a free spread of penicillin in these cases. This does not minimize the gravity of this condition, which will be more fully considered in Part II of this study.

The situation is different if blocks exist within the spinal canal or at the entrance and exit of the fourth ventricle. It is essential to detect a spinal block early, because prompt recognition and the abandonment of the lumbar route for streptomycin administration minimizes its dangers. If a spinal block is not detected early, and lumbar intrathecal administration is continued, then for practical purposes the patient is only receiving intramuscular treatment, which is known to be inadequate. In such cases an alternative route must be found for cerebrospinal administration.

A simple, rapid and reliable test is needed for the early diagnosis of such blocks. The method described, consisting of the use of penicillin as a tracer substance, appears to fulfil those needs and it appears to be better than other methods of diagnosing spinal blocks in cases of tuberculous meningitis. The usefulness of penicillin assay, however, is limited to the investigation of meningitis, when the free spread of antibiotics is in doubt. This method can only detect complete obstruction, and does not exclude the presence of a considerable amount of exudate round the spinal cord or of a tumour not leading to complete obliteration of the spinal subarachnoid space. The limitations of the method are exemplified by the case of an infant with a spinal tumour. Although penicillin passed freely to the cisterna magna after lumbar injection, diodone myelography localized a tumour occupying the segments suggested by the neurological examination. The lumbar/cisternal protein ratio was 15:1, but the block was not complete. In these cases the lumbar/cisternal protein ratio is a more reliable early guide (Ayer, 1922).

The discovery of a block between the cisterna magna and the lateral ventricles implies the presence of at least two large separate compartments requiring cerebrospinal treatment and the total dose must be

divided between them. Ventricular punctures for streptomycin administration and for the relief of pressure are necessary, but the outlook is poor in these cases.

The following criteria are considered to be the indications for performing investigations to exclude spinal block: (i) failure of clinical improvement or deterioration in the patient's condition in the absence of an obvious reason; (ii) unduly low cerebrospinal fluid pressure; (iii) a protein content of the lumbar fluid of over 300 mg. %; (iv) xanthochromia; (v) pain during injection of streptomycin into the lumbar theca; and (vi) persistent backache, girdle pains or pain radiating down the legs, suggestive of involvement of the spinal nerve roots by exudate.

Summary

An attempt was made to determine the optimum route of cerebrospinal administration of streptomycin in the treatment of tuberculous meningitis and to detect a block as soon as possible.

The method employed was a modification of the Oxford technique of the use of penicillin as a tracer substance. It consists of the injection of a standard amount of penicillin into the cerebrospinal circulation at one point and its quantitative detection within 10 minutes from fluid obtained from a different site. This method was used on 55 occasions.

It was found that in the absence of obstruction free and rapid mixing of the penicillin takes place irrespective of the site of its injection. Blocks were detected on 23 occasions, 18 of them within the spinal theca.

The reliability of the lumbar protein content and of the lumbar/cisternal protein ratio as indices of spinal block were compared with the results of the penicillin assay. The former methods were found to be unreliable in cases of tuberculous meningitis.

Penicillin assay is not a suitable method for the detection of tentorial blocks or partial obstructions.

The greatest practical importance of penicillin assay is the detection of spinal blocks because appropriate treatment given in time will minimize its dangers. Spinal blocks are not necessarily permanent.

Indications for the performance of these tests are suggested.

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APPENDIX

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Bacteriological Technique

Since the total cerebrospinal fluid volume varied to some extent, the exact concentration of penicillin in the cerebrospinal fluid expected by complete mixing was unknown. It was therefore only considered necessary to carry out relative assays. The agar-cup-plate method (Fleming, 1942) was adopted.

Method. A layered blood agar plate was prepared, the lower layer consisting of uninoculated nutrient agar and the upper layer of 10% horse blood agar inoculated with a 1/100 dilution of a 24-hour broth culture of Richard's strain of *Streptococcus pyogenes*. Each layer was approximately 2.0 mm. in depth, but as each plate had its own control, an accurate control of the depth of agar was unnecessary. Three cups, 7.0 mm. in diameter, were cut in the agar with a cork borer and sealed with a drop of melted agar. Equal volumes of control and test fluids were placed in the cups:

Cup 1 (Blank Fluid). Cerebrospinal fluid taken before the injection of penicillin.

Cup 2 (Standard Penicillin Solution). A solution of 100 units per ml. soluble penicillin.

Cup 3 (Test Fluid). Cerebrospinal fluid taken after the injection of penicillin.

The blank fluid was a control for bacteriostatic activity due to residual streptomycin. The standard penicillin concentration was chosen at 100 units per ml.

as that of the test fluid was expected to be of this order. The widths of the zones of inhibition of the test organism were measured after 24 hours' incubation at 37° C.

The serum penicillin assays were carried out by a dilution method using whole blood and Richard's strain of *Streptococcus pyogenes* (Emery, Stewart, and Stone, 1949).

Results. The zones of inhibition of the standard penicillin solution varied from 9.0 to 18.0 mm. The control fluids gave maximum zones of 1.0 mm. In 32 cases the test fluids gave zones of from 9.0 to 18.0 mm. and in 21 cases zones of from 0.0 mm. to 1.0 mm. In two cases the blank controls gave no inhibition, the test fluids 3.0 and 4.5 mm. respectively, and the standard solution 10.0 mm.

Blood penicillin assays, carried out on some of the earlier cases to exclude the absorption of penicillin into the blood stream in significant quantity, showed no detectable levels.

Discussion

It is well known that when solutions of an antibiotic are placed in a cup in an agar plate seeded with a sensitive organism, the zones of inhibition produced round the cups are proportional to the concentrations of the antibiotic in the solutions. The actual zone is affected not only by the concentration of the solution, but also by constituents of

the medium, depth of the medium, and size of the cup (Fleming, 1942). These latter factors are constant for any one plate provided that it is poured on a flat surface. Therefore, in the method reported here, the concentration of the test fluid can be compared with that of the standard solution, the latter giving a basis for comparison between different sets of assays. *Streptococcus pyogenes* was chosen as the test organism in preference to *Staphylococcus aureus* since it was found to have a lower sensitivity to streptomycin.

In the 32 cases where the zones of inhibition of the test fluids were between 9.0 mm. and 18.0 mm., the difference between the test fluid zone and the standard zone was never greater than 6.0 mm. representing about 30% of the standard zone. These zones represent a penicillin level of the order of 50-100 units per ml. and are therefore indicative of mixing of the cerebrospinal fluid within the test period.

In the 21 cases where the test fluid zones were 0.0 mm. to 1.0 mm., the blank fluids had given the same degree of inhibition and therefore the results indicated no mixing of the penicillin in the cerebrospinal fluid. In the two cases where the inhibition zones of the test fluids were 3.0 mm. and 4.5 mm. respectively, some slowing down of the mixing was suggested, but not a complete block.

The conclusions drawn on the above criteria have been confirmed by necropsy findings and/or air encephalograms where available.

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