

## SPIROCHÆTOSIS OF THE CEREBRO- SPINAL FLUID.

BY D. O. RIDDEL AND R. M. STEWART, WHITTINGHAM.

IN the period which has elapsed since Noguchi first demonstrated the *Sp. pallida* in the brain of general paralytics, a considerable volume of information has accumulated on the distribution and activity of the parasite within the nervous system. On the other hand, very few reports have been made on the results of search for spirochætes in the cerebrospinal fluid, although the question of their presence there is a subject of more significance than the literature would indicate.

Whatever may be the precise relationship of the cerebrospinal fluid to the brain parenchyma, all investigators are agreed that the two are intimately connected, and it might well be assumed that when *pallida* are present in the nervous system they must exist in the spinal fluid also. It may be that the significance of this relationship has not escaped notice by the pathologist; yet the fact remains that very little in the way of systematic search appears to have been carried out, although at various times it has been found possible to prove the presence of the organism by animal inoculation with cerebrospinal fluid.

Dunlap,<sup>1</sup> in an excellent review of recent studies on spirochætes in general paralysis, states that they have lately been found by Jahnelt in the aorta, and also in the meninges, and he emphasizes the fact that they "have never been seen with the microscope in either blood or fluid except as a possible post-mortem migration into the blood". Several observers have, however, found spirochætes in the spinal fluid in other conditions, though never, so far as we have been able to discover, in large numbers. One of the earliest records of their presence is that of Dohi and Tanake,<sup>2</sup> who found them in the spinal fluid from a case of secondary syphilis where there was no involvement of the nervous system. At a later date Gaucher and Merle<sup>3</sup> reported the presence of spirochætes in ventricular fluid removed post mortem, and in 1910 S ezary and Paillard<sup>4</sup> found a single spiroch ete in the fluid of a case of secondary syphilis. Their patient was a man, age 33, with a papular skin eruption. He developed a total left hemiplegia, and was comatose when lumbar puncture was performed. The spiroch ete was actively motile, regular, and of a

very slender thickness. This appears to be the first recorded case of spirochætal infection of the cerebrospinal fluid in neurosyphilis, and we have been unable to find any other references to this subject in the post-war literature.

It is obvious that until the distribution of the spirochæte in general paralysis has been completely worked out, we are not likely to solve the question of how spirochætes gain access to the brain, and it was a knowledge of this defect that led us to undertake last year a systematic search for their presence in the cerebrospinal fluid. In this communication we propose to give a brief résumé of our results up to the present.

**Methods.**—For the detection of spirochætes we relied on examination of the fluid with a microscope fitted with a dark-ground condenser.\* Search was always made at the earliest opportunity after lumbar or cisternal puncture, and examination of a drop of uncentrifuged fluid was followed up with cover-slip preparations of the deposit after centrifugalization. The chances of finding spirochætes are probably less than when centrifuged fluid is used; but we believe that there is a possibility of so fragile an organism being destroyed during the process, and for this reason we never ran the centrifuge at a high speed.

When living spirochætes were detected in the above way, permanent preparations were made on slides, the fluid being spread in an even film and allowed to dry in the incubator. Of the staining methods devised for demonstrating the organism, we employed either the Giemsa stain or the Fontana-Tribondeau silver process. Films stained by the first method show fewer distortions of the parasite, probably because heat is not essential; but in Fontana's method the hot reagents cause artefacts which interfere with the shape and regularity of the organism. Moreover, we found that in silver-stained preparations the spirochætes always showed an exaggerated thickness. We also succeeded in staining spirochætes by first impregnating the deposit by Jahnel's method<sup>5</sup> and then embedding in wax. In this way very fine sections were obtained, showing the pallida in enormous numbers.

**Material and Results.**—The spinal fluids of 23 cases of general paralysis were used in this investigation: 7 acute cases, and 16 sub-acute or chronic. Included in the series were 3 cases of juvenile paresis.

The results were entirely negative save in one case, so that it will be unnecessary to give a detailed account of the whole series.

---

\* The apparatus used for this investigation was supplied by the Medical Research Council, for whose assistance we wish to express our gratitude.

The only positive result was obtained with the fluid of a juvenile paretic, which showed spirochætes in enormous numbers.

The following is a report of the clinical and laboratory findings:—

**CLINICAL HISTORY.**—E. M. E., age 20, a card-room hand, was admitted to the Whittingham Mental Hospital on June 19, 1922. Her certificate stated: "She is confused in speech, and is constantly removing her night-dress, saying it is wet—this is a delusion. Walks about the ward in a nude condition. Passes urine on the floor."

**FAMILY HISTORY.**—A paternal grandmother of the patient died insane—cause not known. Her father died in an asylum from general paralysis of the insane in 1913. The mother, now an apparently healthy woman, suffered from epilepsy in childhood, and had several still-born children before the birth of E. M. E. and one other daughter.

**PERSONAL HISTORY.**—The patient cut her teeth at 8 months, commenced to walk at 14 months, and to talk at 18 months. In infancy she had measles and bronchitis, but became a fairly robust child. Menstruation did not commence till the age of 18, and was irregular. She was a sober, industrious, quiet, and hard-working girl; reached Standard 7 at school, and earned £2 a week as a card-room operative.

**PRESENT ILLNESS.**—In June, 1921, she was out of work, and about this time her articulation commenced to be faulty and her gait uncertain. In October, 1921, she was placed under medical treatment, and in April, 1922, her mental state became so bad that she had to be removed to the workhouse infirmary.

**State on Admission.**—The patient was a pleasant-looking girl, 5 ft. 2 in. in height, of normal development, and with no obvious manifestations of congenital syphilis. The upper central incisors, though not notched, were slightly peg-shaped. Her thoracic and abdominal organs were normal. Her pupils were unequal, irregular, and sluggish in their reaction to light. The knee-jerks were diminished; other reflexes normal. Articulation was slightly slurring, and gait unsteady. There was no facial tremor, but slight continuous involuntary movements affecting the flexors of the left upper limb were noted. On admission she was confused, restless, and paid little or no attention to questions. Very soon she became more confused, resistive, and destructive. Her habits became very faulty, and she had to be kept permanently in bed. The articulatory defect became more obvious, and teeth-grinding was a prominent feature. After the lapse of several weeks she quieted down, and spent much of her time singing and looking at pictures. The involuntary movements of a clonic type persisted. In September she was too weak to stand or walk, and had an irregular pyrexia which was found to be caused probably by a *B. coli* infection of the urine. She emaciated rapidly. Throughout the period of observation she was extremely emotional, either laughing and smiling, or weeping profusely. Towards the end of the year there was a considerable degree of dementia. Since this date there has been very little change.

**Laboratory Findings.**—The Wassermann reaction was positive in the blood on Sept. 1, and positive in the cerebrospinal fluid on two occasions (Sept. 12 and Nov. 27). Her sister, E. E., gave a negative reaction, but the mother's blood was strongly positive. The patient's blood was twice examined for spirochætes, with negative results.

The following table shows the cerebrospinal-fluid findings:—

TABLE SHOWING RESULTS OF EXAMINATION OF THE CEREBROSPINAL FLUID OF E. M. E.

DATE	SITE OF PUNCTURE	COLLOIDAL GOLD TEST	COLLOIDAL GAMBOGE TEST	CELL-COUNT	ROSS-JONES TEST	SPIROCHÆTA PALLIDA
28/6/22	LP	5555543200	222220	44	+ +	+ +
6/7/22	LP	555554322	222210	—	+ +	+ +
9/7/22	CP	555554322	222210	63	+ +	+ + +
22/7/22	LP	555555543	222110	279	+ + +	+ + +
23/7/22	CP	555555554	222220	119	+ + +	+ + +
8/8/22	CP	555555420	—	89	+ + +	+ + +
15/8/22	CP	555555320	222210	81	+ + +	—
20/8/22	CP	555555310	222220	24	+ + +	—
25/8/22	CP	—	—	9	+ + +	—
1/9/22	CP	5555532000	222110	7	+ +	—
9/9/22	CP	555554320	222110	10	+ +	—
9/9/22 (midnight)	LP	555554432	222210	13	+ +	—
18/9/22	CP	5555410000	222210	5·3	+	—
22/10/22	LP	555554332	222220	12	+	—
6/11/22	CP	5554331000	222210	2	f. +	—
15/11/22	CP	5554410000	222110	3	f. +	—
23/11/22	CP	—	—	2	f. +	—
3/12/22	LP	5555421000	222210	3	+	—

LP=lumbar puncture. CP=cisternal puncture. f. += faintly positive.

*Comment.*—It will be seen that spirochætes were discovered on the first occasion on which lumbar puncture was performed (June 28), and were also found on five subsequent dates (July 6, 9, 22, 23, and Aug. 8). On Aug. 15 none were found, and all further examinations were negative. It therefore seems fair to assume that during a period of at least forty-one days spirochætes were constantly present in the cerebrospinal fluid, and that thereafter they disappeared entirely. It should also be noted that during the period in which they were present there was no cytological or serological peculiarity of the cerebrospinal fluid to suggest their presence. The cell-count lay within normal limits for this class of case, save on one occasion when it jumped from 63 to 279 cells. At first we thought it possible that a high cell-count might indicate an invasion of the cerebrospinal fluid by spirochætes, but a study of other fluids showed that lymphocytes and plasma-cells may be present in very large numbers in fluids which contain no spirochætes. Whether the converse holds—a low cell-count with a spirochætosis—is a question which we are unable to answer, but it is worth noting the very rapid fall in cell-count which followed the disappearance of the organisms in this case.

We are inclined to attribute both the improved mental state and the slow and progressive reduction of cell and globulin contents to the repeated removal of fairly large quantities of cerebrospinal

fluid, and possibly the abrupt disappearance of the spirochætes may have been connected in some way with the spinal drainage.

On twelve occasions we performed puncture of the cisterna magna, for we found that fluid removed from this locality contained more spirochætes than the lumbar fluid. A rough estimate of the total number of spirochætes in films prepared from the cisternal and lumbar fluids showed a striking difference. Thus, on July 6 only three spirochætes were found in a stained lumbar film, whereas three days later fifty-four were counted in a drop of equal volume of cisternal fluid, and it seems hardly likely that the difference could be due to a pullulation of spirochætes in the two days which intervened between the punctures.

Attempts were made to cultivate the organism, and for this purpose we employed Noguchi's agar-tissue medium,<sup>6</sup> Muhlens and Hoffmann's horse-serum agar,<sup>7</sup> and a medium prepared by using equal parts of hydrocele fluid and the patient's cerebrospinal fluid. In spite of strict anaerobic conditions, the results were uniformly negative. Arrangements were then made for animal inoculation, for, as we were dealing with a heavily-infected fluid from a juvenile parietic whose father had succumbed to general paralysis, it seemed an excellent opportunity of repeating the work of Levaditi and Marie,<sup>8</sup> who believe in the existence of a neuropathic strain. Unfortunately the unexpected disappearance of the organism put an end to this part of our investigation.

As the *Spirochaeta pallida* appears to be distributed in enormous numbers throughout the body in congenital syphilis, their discovery in the cerebrospinal fluid from a case of juvenile paresis suggested that an infection of the cerebrospinal fluid might be a distinctive feature of this form of dementia paralytica; but such does not appear to be the case, for we were unable to find any spirochætes in two other cases.

**Morphology.**—Under dark-ground illumination the spirochætes appeared actively motile, moving rapidly across the field, and exhibiting the typical corkscrew movement. For the most part they varied extremely little in form, twelve being the average number of spirals, all perfectly regular, well marked, and closely resembling each other. Occasionally very much longer forms were seen, and these gave the impression of being rather more slender than the shorter forms. Movement continued for as long as six hours, although actual translation across the stage usually ceased in two or three hours. We had no means of preserving a uniform temperature of the microscope stage, but the heat generated by the Liliput arc lamp was sufficient to prevent the slides from becoming cold. In cerebrospinal fluid preserved in the incubator at 37° C. under ordinary aerobic conditions,

the spirochætes continued to show movement for twelve days after withdrawal of the fluid. At no time were we able to observe evidence of longitudinal division.

Another interesting feature was the presence of peculiar round bodies. These, frequently terminal or attached to one side of the organism, were in size not very much greater than the transverse diameter of the pallida. Their refractive power appeared to be higher than that of the body of the organism, and gave the impression of a brilliant 'head lamp' as the spirochæte moved rapidly across the field. Noguchi<sup>9</sup> has also remarked the presence of similar spore-like bodies, whose significance is quite unknown. Long after the

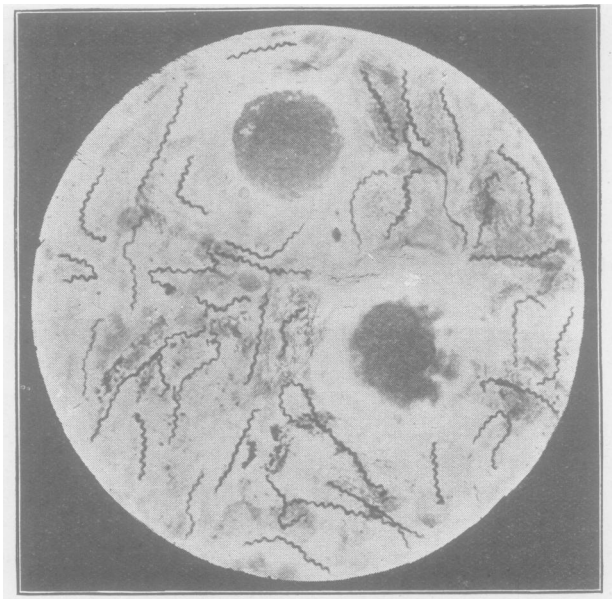


FIG. 1.—*Spirochæta pallida* in cerebrospinal fluid. Fontana stain ( $\times 1200$ ). Two cells are seen, one a lymphocyte and the other a polymorph.

disappearance of spirochætes we found minute spherical bodies indistinguishable from those attached to the pallida.

In stained films very similar morphological characters were observed; but the spirals were often irregular and somewhat flattened out, as though the organisms had become stretched in the process of staining.

It was an easy task to count as many as 100 pallida in a drop of uncentrifuged cerebrospinal fluid  $\frac{1}{15}$  c.c. in volume. (We used Donald's<sup>10</sup> drop-method of counting.) The illustration accompanying this paper (*Fig. 1*) was obtained by taking separate microphotographs

of some forty spirochætes from a single film preparation; the prints were then rephotographed to give a composite picture, and we would like here to acknowledge our indebtedness to Mr. A. H. Fann, Chief Laboratory Assistant at the Whittingham Mental Hospital, who prepared the illustration.

#### SUMMARY.

The spinal fluids of 23 cases of general paralysis were examined for the presence of *Sp. pallida*. Twenty-two fluids were negative; but in one, obtained from a case of juvenile paresis, enormous numbers were found. Their presence in the cerebrospinal fluid was verified on six consecutive occasions, which covered a period of forty-one days. Thereafter they disappeared, and could not be again identified, although twelve more punctures were performed. Attempts at cultivation were unsuccessful, but the motility of spirochætes preserved *in vitro* was retained for twelve days.

Fluid removed by cisternal puncture contained more pallida than were seen in lumbar cerebrospinal fluid. Peculiar spore-like bodies attached to the ends or sides of the organisms were seen with the aid of dark-ground illumination, and similar bodies continued to exist in the fluid when the spirochætes had completely disappeared.

---

#### REFERENCES.

- <sup>1</sup> DUNLAP, *Arch. of Neurol. and Psychiat.*, 1922, viii, 589.
- <sup>2</sup> DOHI and TANAKE, *Japan Zeit. f. Derm.*, 1905, v, 537.
- <sup>3</sup> GAUCHER and MERLE, *Comptes rend. de l'Acad. des Sciences*, 1909, March 29.
- <sup>4</sup> SÉZARY and PAILLARD, *Soc. de Biol.*, 1910, lxxviii, 295.
- <sup>5</sup> JAHNEL, *Arch. of Neurol. and Psychiat.*, 1922, viii, 349.
- <sup>6</sup> NOGUCHI, *Jour. of Exper. Med.*, 1912, xv, 90.
- <sup>7</sup> HOFFMANN, *Deut. med. Woch.*, 1911, 1546.
- <sup>8</sup> LEVADITI and MARIE, *Ann. de l'Inst. Pasteur*, 1919, xxxiii, 741.
- <sup>9</sup> NOGUCHI, *Jour. of Exper. Med.*, 1911, xiv, 199.
- <sup>10</sup> DONALD, *Rev. Neurol. and Psychiat.*, 1914, xii, 333.