California MEDICINE

OFFICIAL JOURNAL OF THE CALIFORNIA MEDICAL ASSOCIATION

Volume 89 DECEMBER 1958 Number 6

The Spirochete and Multiple Sclerosis

HENRY W. NEWMAN, M.D., CAROLA PURDY, M.D., LOWELL RANTZ, M.D., and F.C. HILL, JR., M.D., San Francisco

• Cultures on anaerobic medium were made of the spinal fluids of 27 patients with multiple sclerosis and 13 controls after the method described recently by Ichelson. Where Ichelson found organisms resembling spirochetes in 78 per cent of patients with multiple sclerosis, we found some form of what appeared to be a living micro-organism in 18.5 per cent. The control fluids were all sterile. The work requires confirmation and amplification.

IN SPITE of the concerted efforts of many researchers in the field of multiple sclerosis, the cause of this common neurological disease remains obscure. Among the great variety of agents suspected at one time or another of being the causative factor, a living organism bearing a resemblance to a spirochete has been implicated by a number of workers. In 1921 Speer[3] made a darkfield preparation by wiping the surface of a plaque from fresh autopsy material with a sterile loop and saw motile spiral organisms. A few years later Schroeder[2] reported finding black spiral fibrils, stained by the silver method of Levaditi, in lesions of two patients dying of the disease; he was unable, however, to find any spirochetes in another patient with multiple sclerosis nor in ten controls free of the disease, and noted that, in the absence of culture and transmission, Koch's postulates were not fulfilled. Steiner[4] maintained his enthusiasm for the spirochete as the cause of multiple sclerosis longer than most investigators, but no convincing demonstration of production of nervous system disease by the organisms said to have been isolated from human lesions was ever presented.

After a considerable period in which interest in this field had lagged, Ichelson[1] in 1957 reported striking results in the culture of a spirochete-like organism in an anaerobic medium. These organisms grew on cultures of spinal fluid in 78 per cent of 76 cases of patients with multiple sclerosis; they did not grow on cultures of spinal fluid from 28 persons who did not have the disease. The illustration of the organism in that report, a silver stained specimen, showed rather tightly coiled spirals looking much like the spirochete of syphilis. The organisms previously described by other workers, as observed in fresh preparations, had shown a variety of forms, some spiral, others more like spermatozoa or a tennis racket. Ichelson's most interesting report encouraged us to try to repeat the work, following as closely as possible the details of procedure as set down in the communication.

METHOD

The medium was prepared exactly as described by Ichelson,[1] the same brand of materials being used where brands were specified. Difficulty was encountered in filtering the medium through a Selas filter, which became plugged after approximately 30 cc.

From the Division of Neurology and the Laboratory of Bacteriology, Department of Medicine, Stanford University School of Medicine, San Francisco, California.

Presented before the Section on Neurology and Psychiatry at the 87th Annual Session of the California Medical Association, Los Angeles, April 27 to 30, 1958.

was passed. Since filtering the medium through filter paper before Selas filtering was attempted did not obviate this difficulty, a Seitz filter was substituted for the Selas filter, with satisfactory results.

Sterility tests of the medium were made in thioglycollate broth and blood plates, aerobically and anaerobically, at room temperature, at 30°C and at 37°C. The human serum was drawn on the day of preparation of the medium. The rabbit serum for the first 20 specimens had been stored in deep freeze; that used subsequently was obtained on the day of preparation of the medium. The medium was stored at room temperature. It was not renewed at regular intervals for the inoculation of the first half of the series of spinal fluids. When it was recognized that possibly anaerobic conditions were not maintained over a prolonged period, fresh medium was prepared at least every three weeks.

Specimens for phase microscope examination were obtained at weekly intervals by inserting a capillary pipette through the vaseline-paraffin oil layer at the top of the culture tubes to the bottom of the tube and withdrawing 0.3 cc. of culture medium. A drop of this fluid was placed on a slide and polystyrene latex beads, diameter 0.814 micron, were added for the adjustment of the fluid level under the phase microscope. A cover-slip was placed on top of this preparation and examination under oil immersion (magnification ×1280) was carried out.

Blanks, consisting of saline solution inoculated into the culture medium, were similarly prepared and examined.

Positive cultures were transferred to fresh culture media by withdrawing 0.5 cc. of culture medium from the bottom of positive culture tubes with a capillary pipette.

Between September 1957 and June 1958, 42 specimens of spinal fluid were collected under sterile conditions from 40 patients, thirteen of whom were controls known not to have multiple sclerosis, and 27 patients with a secure diagnosis of multiple sclerosis. From one patient in each group two specimens were taken at about a month apart. The fluid was added to the culture medium within two to three hours of being drawn, except in one instance in which the fluid was allowed to stand overnight. The bacteriologist was unaware of whether the fluid was from a patient who had multiple sclerosis or from a control until after the study was completed.

RESULTS

Of the 42 spinal fluid specimens tested, six contained organisms. All the positive specimens were from patients with well documented multiple sclerosis. Both specimens from the patient who was twice tested were positive. None of the controls and none of the blanks contained these forms. Various forms of motile micro-organisms were observed. In length they varied from 3 to 10 micra and in width from 0.7 to 0.9 micra. A few organisms looked like a curved rod with two flagellae, while others were short and plump. No spirals were seen in this fresh material, but the appearance was not unlike that described in the early reports. As described by Ichelson, they seemed to "dive into the fluid," and their progress across the field was quite different from the Brownian movement of the polystyrene beads. The organisms appeared in the medium in from four to twenty-four days, and they were never numerous, two to three being the average number found in a single preparation. After three to five weeks all positive cultures showed non-motile debris similar in shape to the motile forms previously seen; such debris was never seen in negative cultures whether from persons with the disease or from controls. The gross appearance of the positive cultures was clear, and they could not be distinguished from the controls on this basis. All the positive cultures could be transferred once to new culture medium, but not further; thus it cannot be stated definitely that multiplication occurred in the sub-culture, as the organisms in the second culture might have been only those surviving from the original culture.

TABLE 1.—*Clinical Data on Patients with Multiple Sclerosis with "Positive" Cultures*

Sex	Age at Onset	Time Since Onset	Spinal Fluid Protein Mg. per 100 cc.	Colloidal Gold Test Reaction
Male	33	30 years	58	5444321000
Female	33	4 years	44	5544321000
Male	42	7 years	64	5554321000
Female	20	3 weeks	22	1111100000
Male	30	3 weeks	84	0012221000
		7 weeks	42	0112221100

Table 1 shows some features of the cases in which there were positive cultures, five in number with two specimens of spinal fluid from the fifth case. It will be noted that in three of the patients the disease was of considerable duration; and these three had a first zone reaction to the colloidal gold test. In two quite recent cases the colloidal gold test curves were flatter. In general, it may be stated that there was no consistent difference clinically between cases in which cultures were positive and those in which they were negative, yet in all the "positive" cases the disease was either quite acute or the spinal fluid findings were of an acute nature.

CONCLUSIONS

In an appreciable proportion of our small series of multiple sclerosis patients (5 of 27, or 18.5 per

cent) some form of what seemd to be a living organism was grown on an anaerobic medium as described by Ichelson,[1] while no such organisms were found in a series of patients without the disease. This is a much smaller percentage than reported by Ichelson, but not out of line with data in point reported by earlier investigators, as summarized by Steiner.[5] All the patients with "positive" cultures were either in an acute early phase of the disease or had a colloidal gold test curve in the first zone. The exact nature of the organisms, if such they be, remains unknown and further study of their cultural and staining characteristics, as well as the trial of inoculation into experimental animals to try to reproduce the disease, seems urgently indicated. It is hoped that our incomplete but possibly significant findings will stimulate further work on this almost forgotten aspect of the etiology of multiple sclerosis.

Stanford University Hospital, San Francisco 15 (Newman).

REFERENCES

1. Ichelson, R.: Cultivation of spirochete from spinal fluids of multiple sclerosis cases and normal controls, Proc. Soc. Exper. Biol. & Med., 95:57, 1957.

2. Schroeder, G. E.: Sclerose en plaques et spirochetes, Rev. Neurol., 41:785, 1924.

3. Speer, E.: Spirochätenfund im menschlichen Zentralnervensystem bei multipler Sklerose, Münch. Med. Wchschr., 68:425, 1921.

4. Steiner, G.: Demonstration von Spirochäten im menschlichen Gehirn bei multipler Sklerose, Deutsche Ztschr. f. Nervenheilk., 107:112, 1928.

5. Steiner, G.: Acute plaques in multiple sclerosis, their pathogenetic significance and the role of spirochetes as etiological factor, J. Neuropath. & Exper. Neurol., 11:343, 1952.