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Cultivation of Spirochaetes from Spinal Fluids of Multiple Sclerosis Cases and Negative Controls.* (23117)

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Since 1838 when multiple sclerosis was first recognized as a demyelinating disease, many attempts have been made to establish its etiology. There were many conflicting theories. Originally it was thought that multiple sclerosis was due to toxins that destroy the myelin sheaths; second, that it was an allergic or biochemical reaction. Buzzard(1) advanced the theory that due to resemblance of multiple sclerosis to cerebrospinal syphilis, the causative agent may be an organism of the Treponema family. He did not succeed in demonstrating the suspected organism. Steiner and Kuhn(2) found spirochaetes in brain tissues and spinal cords of patients who had died of multiple sclerosis. Adams, Blacklock and M'Cluskie(3) were able to demonstrate spirochaetes under darkfield illumination while examining fluid from lateral ventricles of monkeys and rabbits injected with material from multiple sclerosis cases. This fluid was examined several months after inoculation when the animals developed signs of illness. The spirochaetes were identical in appearance with those described by Steiner. All attempts to cultivate the spirochaetes failed. Collins and Noguchi(4) tried to grow the organism by inoculating spinal fluids from multiple sclerosis cases into Noguchi culture medium. They were unable to get positive results. In 1951, I devised a culture medium in which spinal fluids from known cases of multiple sclerosis were inoculated, and, after one to 2 weeks incubation, few spirochaetes were found under phase and darkfield illumination. Cultures from spinal fluids of healthy individuals and of patients with other neurological diseases were negative and remained sterile after one year's incubation. The original culture medium did not show many organisms per field; growth was poor. To obtain a better growth, the culture medium was modified, and subcultures from the original positive culture and fresh spinal fluid reinoculated into the modified medium showed heavy growth of spirochaetes after several days' incubation.

Methods. Stoppered test tubes are sterilized by autoclaving, then sterilized again in hot air sterilizer for one hour at 180°C. About 10 ml of spinal fluid are collected into

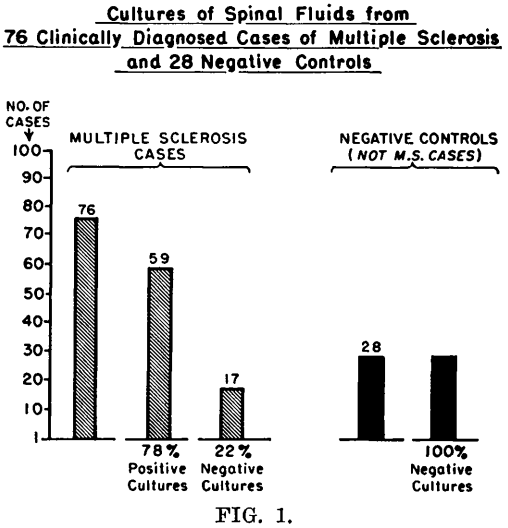

FIG. 1.

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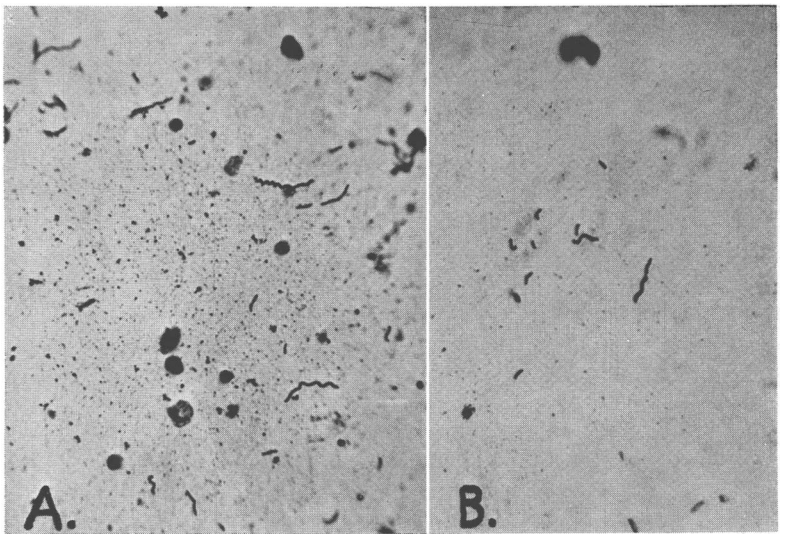

FIG. 2. Photomicrographs of 2 fields, A and B, from a smear of culture of spinal fluid from M.S. case A.M. after staining by the silver nitrate-tannin method. Magnification × 970.

cooled sterile tubes and centrifuged at 1500 rpm for 1½ to 2 hours. If red blood cells are present or the fluid is xanthochromic, it is not suitable for cultivation. The supernatant fluid is discarded, leaving a few drops mixed with sediment and, by a Wright pipette, inoculated into the following modified culture medium: Distilled water 1000 ml. Brewers Thioglycollate Medium (Difco #B236) 20 g. Bacto-Asparagin (Difco) 0.4 g, L-Cystine (Difco) 0.2 g. Bacto-Peptone (Difco) 1 g. Boil 20 minutes, cool to 37°C. add—rabbit sera 20 ml. Wassermann negative human sera 60 ml. Filter through Selas 03 filter, pour into sterile test tubes and inoculate with spinal fluid as described above. To secure anaerobiasis, about 3-5 ml of sterile paraffin oil (50%) and yellow vaseline (50%), previously sterilized one hour in autoclave and cooled, are poured into the inoculated tubes. Inoculated cultures are incubated at 30°C and examined once a week under phase microscope. Silver nitrate-tannin method is used for staining smears from positive cultures.

Results. The results obtained by culturing the spinal fluids from 76 cases of clinically diagnosed multiple sclerosis and 28 individuals not recognized as having multiple sclerosis are summarized in Fig. 1.

Morphology. By phase- and darkfield-microscopy, micro-organisms are observed which vary from 10-22 μ in length, about 1 μ thick. They have wide spirals; some have a loop at one end while others resemble a tennis racquet. Photomicrographs (2a and 2b) of stained organisms are reproduced in Fig. 2. They are actively motile. They also have a tendency to dive into the fluid. The spirochaetes are identical in appearance to those found in brain tissue by Steiner, who named them Spirochaeta Myelophthora.

Summary. New culture medium was devised in which it was possible to grow spirochaetes from spinal fluids of cases of multiple sclerosis. Photomicrographs of the cultured spirochaetes are reproduced. The results of cultivation are given in Fig. 1.

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