rums contain the major portion (50% or more) of the niacin of the parent serum which had been extensively dialyzed.

Summary. The essential growth factors associated with serum proteins are present in cold ethanol fraction IV-4 for most human serum pools and in fraction II + III for 2 individual equine serums. Cytotoxic activity has been found in fraction II + III from most human serum pools. Further subfractionation of IV-4 and II + III by the established cold ethanol methods fails to yield active subfractions. The significance of this finding is discussed.

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Received July 23, 1959. P.S.E.B.M., 1959, v102.

Is Multiple Sclerosis Caused by *Spirochaeta myelophthora* (Steiner)?* (25197)

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Ichelson(1) reported regular cultivation of a spirochete resembling *Spirochaeta myelophthora,* previously described by Steiner(2). She claims to have isolated from spinal fluid of 78% of 76 persons with clinical diagnosis of multiple sclerosis, a large spiral organism morphologically resembling members of the genus, Borrelia. In contrast she was unable to obtain a single positive culture from spinal fluid of 28 persons with diagnoses of other illnesses. We believed that several aspects of this report demanded clarification and/or confirmation. First, the report that one can cultivate "Borrelia-like" organisms from 78% of the spinal fluid of persons with multiple sclerosis regardless of stage of disease should be confirmed. Secondly, no exact data were included in the original report as to time following inoculation when each culture first be-

* Supported by grants from Multiple Sclerosis Fdn. of Am., Lewis J. Pollock, Responsible Investigator.

came positive. A third point open to discussion is the method of inoculation. In the illustrations accompanying the article by Ichelson are seen organisms which resemble some of the large Borrelia species resident in mouths of normal individuals. According to the author, inoculation of spinal fluid was made by blowing it into the medium with a Wright pipette. Since spirochetes are able to pass bacteriologic filters at times, could cultures have been established by passage of spirochetes through the pipette plugs by the force of blowing? This report presents results of our attempt to confirm Ichelson.

Materials and methods. The medium employed(1) contains Brewer's thioglycollate, asparagin, L-cystine, peptone, Wassermann-negative human serum, and rabbit serum, in a water diluent. This preparation was sterilized by filtration through a Selas 03 filter and was dispensed into tubes. Into the bottom of a tube of this medium there was delivered a few drops of the centrifugate from 10 ml of patient's spinal fluid. The inoculated tube then was overlaid with a paraffin oil-vaseline mixture. The tube was incubated at 30°C and examined once a week until growth took place. Two batches of medium were prepared adhering as closely as possible to Ichelson's(1) published methods. It was found impossible to prepare the medium as described by this worker, which was also the experience of others(3,4). Presence of agar in the thioglycollate broth caused a rapid plugging of pores of the Selas 03 filter. Consequently the Wassermann-negative human serum and aseptically-removed normal rabbit serum were filtered separately through such a sterile filter. The remainder of the ingredients were then prepared according to her directions, passed through sterile warmed M and UF grade sintered glass filters and then added aseptically to the serum mixture. Finally, all tubes of media were incubated for sterility for 3 to 5 days in the 37°C incubator and remained at room temperature for a week before inoculating them. Neurologists associated with Dept. of Neurology and Psychiatry, Northwestern University Medical School, obtained specimens of spinal fluid from patients who were either ambulatory or hospitalized at the affiliated hospitals of Northwestern University Medical School. Specimens were drawn aseptically into sterile, screw-capped tubes. Each specimen was kept at body temperature until delivered to the laboratory, the time between withdrawal of spinal fluid and its arrival at laboratory not exceeding 2 hours. Each specimen was transferred at once to a chemically clean, sterile, centrifuge tube and centrifuged at 1500 rpm for 2 hours (following Ichelson's procedure). One drop from the original sample tubes was examined immediately under a darkfield microscope for any animate or inanimate particles that might have some bearing on the problem. Such examinations were made with great care, by scanning many fields of each preparation. After 2-hour centrifugation, most of the supernatant fluid was removed, leaving only enough to allow for mixing with sufficient sediment to inoculate tubes and to make 3 additional slide preparations. One of these latter was examined by darkfield microscope, the second and third allowed to dry, stained by Gram and Giemsa methods respectively, and then examined. Inoculations were made into 3 tubes of broth-serum culture medium of Ichelson (2 tubes of one batch and 1 tube of a second batch of medium), and into 2 tubes of trypticase soy broth. The first serum-broth tube was inoculated by blowing the spinal fluid with the mouth from the pipette into base of the tube. The second was inoculated in the same manner, except that a bulb-manipulated pipette was used. A fresh bulb-manipulated pipette was substituted to inoculate the third tube. The trypticase soy tubes were inoculated, also, with a bulb-manipulated pipette. All serum-broth cultures were incubated at 30°C. One trypticase soy culture was left at room temperature, the other incubated at 37°C. These latter were intended to serve as sterility controls of cultures. Positive control tubes of serum-broth media were inoculated from stock culture of *Leptospira icterohemorrhagiae* (obtained from Dr. Evelyn B. Tilden, Brookfield Zoological Gardens, Brookfield, Ill.) to be sure that the medium would support growth of a known strain of spirochete. Since Ichelson did not state the total time over which she incubated her cultures, our cultures were kept for at least 6 months, examined at weekly intervals

for appearance of growth; grossly by transmitted light, and microscopically by darkfield method.

Results. Forty-two patients have been examined, 28 of whom are afflicted with multiple sclerosis, the remaining 14 with other conditions. It seems pertinent that some evidence be presented in support of the diagnosis of multiple sclerosis in these 28 patients. All were examined by an experienced Board certified neurologist(5). Of 28 patients listed as suffering from multiple sclerosis, 3 had a presenting syndrome of retrobulbar neuritis. Twenty-three were considered to have a progressive type of illness. Of 28 patients, 50% or more exhibited the following signs and symptoms: pyrimidal tract involvement, temporal pallor, nystagmus, bladder disturbance, ataxia, vibratory sense disturbance, diplopia, and position sense disturbance. Eleven of the group had speech difficulties. Twenty-four of these persons were between the ages 20-40 years; only 3 were in the 41-50 age group.

In 2 instances only did growth occur after inoculation of media previously described. This was probably contamination, since all inoculated tubes of the first specimen showed presence of Gram-negative bacilli; while the second revealed Gram-positive bacilli, along with minute amounts of broken cork pieces from the specimen tube cap lining. No spirochetes of any type were seen in any tube inoculated, except in the spirochete-positive control tubes.

Comparisons of examinations by darkfield, of material taken immediately from the specimen as soon as it reached the laboratory, and from material after it had been centrifuged for 2 hours, showed some variation. In some instances there were variable amounts of erythrocytes. The main difference in the centrifuged specimens (2 hours) consisted of the finding of leucocytes, which also differed in number from one, found after examining the whole specimen, to many/high power field.

The interesting thing about these white cells was their reaction to unfavorable environment, evidenced by their sending out various types of filipodia and/or pseudopodia from cell periphery. Sometimes the protoplasm would extend outward in very long strands, with a length of 10 to 15 times the diameter of the cell. These would wave about actively, eventually becoming pinched off in a manner such as to give the strand the appearance of an irregular string of beads. Some would break off from the parent cell and float about, disintegrating into smaller pieces, especially after pseudopodia became detached from the cell. Most of the movement was due to molecular motion of the preparation fluid. These bits of protoplasm appeared as brilliant white vibrating pieces which could very easily have been mistaken for a spirochete had not their origin been observed.

Erythrocytes usually appeared with many short extensions, about one-third the cell diameter, which gave them the appearance of being ciliated. These tiny filipodia were very mobile while attached to the cell.

The Gram stained films revealed nothing that was not already seen by darkfield examination; the Giemsa stained preparations showed leucocytes to be mostly lymphocytes, with only an occasional polymorphonuclear leucocyte. Eighty-six %(24) of the spinal fluids from cases of multiple sclerosis contained leucocytes which showed changes as described above. Of 14 spinal fluids from patients with diseases other than multiple sclerosis, only 5, or 25%, revealed such cells or their components. Patients in this group had been diagnosed as suffering from various disturbances such as cerebral vascular accident, Friedreich's ataxia, post-infectious polyradiculo-neuritis, psychomatic epilepsy, myasthenia gravis, hypertrophic osteoarthritis, and CNS lues. That our results might differ from those of Ichelson(1) so markedly by chance alone is less than one in a thousand when measured by the X^2 Method.

Summary. Using technics very similar to those proposed by Ichelson, a study was made of 28 persons with well documented diagnosis of multiple sclerosis, and 14 persons with other conditions which involved the central nervous system. We attempted to isolate a spirochete similar to the one which Ichelson claims to have found. We were unable to confirm her work. The possibility that this result could have occurred by chance alone is statistically invalid.

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Received June 1, 1959. P.S.E.B.M., 1959, v102.

Electrophoretic and Immunologic Studies of Rivanol-Fractionated Serum Proteins.*† (25198)

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Rivanol (6,9 diamino-2 ethoxyacridine lactate) has been employed by Hořejsí and Smetana(1,2) for precipitation of various protein fractions from serum or plasma at room temperature and in slightly alkaline solution. In contrast to most other procedures, *e.g.*, alcohol, salt fractionation, etc., addition of rivanol to serum does not result in precipitation of gamma, and to a lesser extent, beta globulins. This unique property of rivanol has been utilized in simple methods for preparation of highly purified gamma globulin(2), for separation of beta-1-metal combining globulin(3) and preparation of ceruloplasmin from Cohn's fractions III and IV(4). The apparent contradiction of the original concept of rivanol as specific reagent for separation of gamma globulin from all other serum protein fractions (2) and its use by others in preparation of beta globulin components led to the present study of rivanol-serum protein interactions. Our studies demonstrate that the supernatant solution, following rivanol precipitation of more rapidly migrating electrophoretic serum protein fractions, contains some beta globulin in addition to gamma globulin, as shown by both paper and moving boundary electrophoresis. In addition, this rivanol-containing supernate may be injected intravenously into rabbits resulting in antihuman-globulin sera of relatively high titer.

Materials and methods. Rivanol. Preparation of rivanol in these studies was obtained from Special Chemical Dept. of Winthrop Labs. under trade name "Ethodin." (Lot #N325FA). A 0.4% solution of rivanol in distilled water was prepared. *Rivanol-serum preparations.* To one volume of normal, human serum were added 1,2,3,5 and 9 volumes of 0.4% rivanol solution. The mixtures were left overnight at 4° and the resulting supernates were separated from precipitates for each mixture by centrifugation at 2250 g (International Type II) for 10 minutes. Supernates were then analyzed for protein composition by paper electrophoresis as described below. Corresponding precipitates were dissolved in 2 ml of citrate buffer (pH 5.0, 0.2 M). Except for small amount of insoluble material, the remainder of precipitate was readily soluble in this buffer. The redissolved precipitates were then subjected to paper electrophoresis in the same manner as their corresponding supernates. *Paper electrophoresis procedure.* The free-hanging horizontal strip technic of Dettker and Anduren(5) was used for paper electrophoresis determinations. The technic was essentially the same as described by Block, *et al.*(6), using Whatman No. 1 paper, barbital buffer (pH 8.6, 0.05 ionic strength) 220V for 6 hours and staining with Amido-Black 10B. Multiple aliquots of 10 lambda in inverse proportion to protein content were applied to paper strips (Fig. 1).

* Aided by Grant from U.S.P.H.S.

† The authors wish to acknowledge the aid of Shirley Gerstenfeld in the paper electrophoretic patterns, of Francis Vecsler, in performing moving boundary electrophoresis experiments, and Lillian Salowitz in editing and typing of manuscript.