Paper Chromatography of Blood Plasmas in Multiple Sclerosis.*† (18431)

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(Introduced by W. G. Penfield.)

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Previous observations have indicated the possibility that the degree of severity of multiple sclerosis is related directly to the amount of the daily fat intake(1). This observation has stimulated studies of the effects of high fat meals on the composition of the blood of normal men and of dogs. First, it was shown that 6 to 9 hours following high fat meals the red blood cells and chylomicra in normal humans and dogs have a tendency to aggregate(2). Second, these changes in the physical stability of the blood were found to be accompanied in the dog by alterations in the plasma protein pattern of the paper chromatogram(3).

In the present study it will be shown that with the paper chromatographic technic(4) it is possible to demonstrate significant changes in the plasma proteins of patients with multiple sclerosis.

Material and methods. Plasmas were obtained from a group of intensively studied patients with multiple sclerosis, who were followed at the Neurological Clinic of the Royal Victoria Hospital. All bloods were obtained from fasting patients and were prevented from clotting by heparin sodium salt (Connaught Laboratories). The bloods were obtained at different intervals of time, and when possible, during exacerbations and remissions of the disease. From 1 to 8 tests were made on each patient. All of the 26 patients had well established disease; most of them had been admitted to the Montreal Neurological Institute for diagnosis and treatment; and all but one were ambulatory. Some of the patients were on low fat diets currently being used in this clinic to treat patients with multiple sclerosis. A total of 11 normal subjects was also studied. Three of these were studied at weekly intervals over a period of 6 weeks, and as a control for those multiple sclerosis patients receiving the low fat diet, these 3 subjects were put on the same low fat diet for a period of 3 weeks. The capillary ascent method was used in chromatographic analysis(5). Whatman No. 1 filter paper was used exclusively in this work. Seven sets of conditions were used to examine the plasma of each normal and each patient. One condition did not include the surface active agent, and the others used 0.02, 0.04, and 0.06 ml Tween 81, and 0.01, 0.03 and 0.05 ml Tween 85 per 0.5 ml of the test plasma.§ The illustrated chromatograms show increasing concentrations of Tween 81 in the top row and Tween 85 in the bottom row. Mixing is easier if the surface active agent is placed in a small culture test tube first, then 0.5 ml of plasma is added and these are thoroughly stirred with a glass rod. Then 0.02 ml of 0.3% hemin is added, and aliquots of the resulting plasma solution (0.02 ml) are placed in the lower left hand corner of 8″ x 8″ Whatman No. 1 filter paper. This is allowed to dry, and the filter paper is stapled in the form of a cylinder, taking care to keep the edges from touching. The cylin-

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2. Swank, R. L., *Am. J. Physiol.,* in press.

3. Swank, R. L., Franklin, A. E., and Quastel, J. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 850.

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5. Williams, R. J., and Kirby, H., *Science,* 1948, v107, 481.

§ We are indebted to the Atlas Powder Co., Wilmington, Del. (per G. F. Sterne & Sons, Brantford, Ont.) for samples of Tween 81 (Polyoxyethylene sorbitan monooleate) and Tween 85 (Polyoxyethylene sorbitan trioleate).

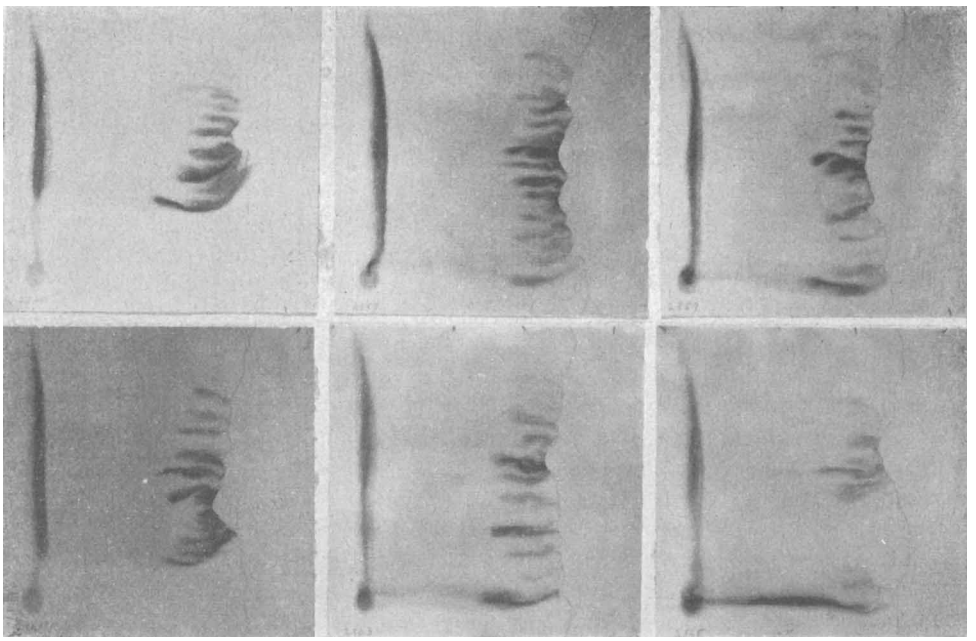

FIG. 1.

Shows 1 chromatogram of a series of 6 from a normal woman 33 years of age. The illustrated chromatograms show increasing concentrations of Tween 81 (.02, .04, and .06 ml per .5 ml of plasma) in the top row, and increasing concentrations of Tween 85 (.01, .03, and .05 ml per .5 ml of plasma) in the bottom row.

ders are placed standing in a pyrex dish containing 100 ml M/10 sucrose, and covered with a bell jar, and the solvent is allowed to reach the top of the paper. This requires about 1-1½ hours. The cylinders are air-dried, unstapled, and restapled at 90° to the original direction. They are again placed erect in a pyrex dish which contains 100 ml M/10 NaK tartrate, and the solvent allowed to go about two-thirds up the paper. After drying, the papers are streaked with the benzidine reagent, as described previously(4). The chromatograms are photographed after a slight delay (about a minute) to allow full color development, and before the characteristic brown background appears.

Results. Protein patterns observed in healthy, young adults. Fig. 1 shows 1 chromatogram of a series of 6 taken at weekly intervals from a healthy woman 33 years of age. The protein pattern in this subject varied very little from week to week during this period. The maximum variation consisted of an absence of several central fractions in the chromatogram obtained when 0.03 ml Tween 85 was added to the plasma. Fig. 2 shows 1 chromatogram of a series of 6 at weekly intervals from a healthy man 29 years of age. Even greater consistency of the protein pattern was shown in this series of tests. Nine other controls (6 women, 3 men) were examined. They showed similar protein patterns with no great variations upon repeated testing. When 0.05 ml Tween 85 was used with the plasma, few protein fractions were observed in either the control or experimental subjects.

The chromatograms of normal, healthy persons appear to vary to some extent from time to time. The variations, however, the causes for which are as yet unknown, have not affected the general protein pattern as observed in normal individuals. Generally speaking the normal pattern indicates the presence of a large number of fractions in all conditions of examination with the exception of the higher concentrations of surface active agents (see Fig. 1-2). It is possible

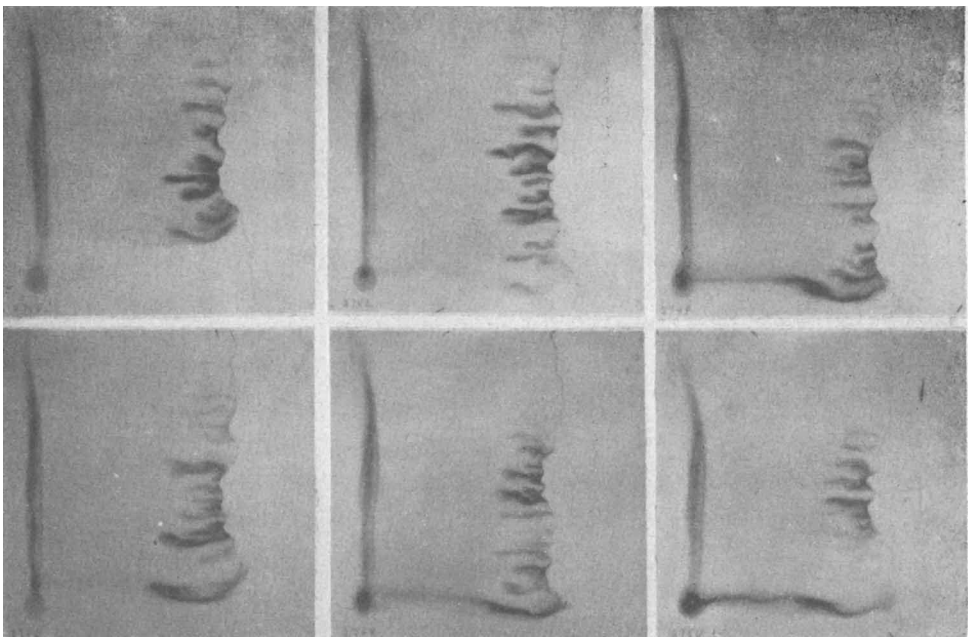

FIG. 2.
Shows 1 chromatogram of a series of 6 from a normal male 29 years of age.

that in normal women these variations are greater than in men, but insufficient material prevents any conclusions on this point. The relationship of the menstrual cycle to the plasma proteins may be of importance in the apparent greater variation observed in women, but this has not been studied.

Protein patterns observed in multiple sclerosis patients. Of a total of 81 chromatograms from 26 patients with multiple sclerosis, 31 tests in 17 patients showed distinct abnormalities, and 1 or more tests in each of 4 patients indicated probable abnormalities. In 5 patients all the chromatograms were normal, despite the occurrence in 2 of them of mild exacerbations of disease during the period of study. In one of the latter 2 patients the chromatograms were carried out 4 days after, and in another case one day after the onset of mild retrobulbar neuritis (as evidenced by a sudden partial loss of vision with return to former vision in about 2 weeks).

In 3 patients striking changes were observed in the chromatogram during exacerbations of disease. These changes were preceded by normal (2 cases), or nearly normal (1 case) chromatograms, and were followed by a return of the chromatogram towards (or to) normal during the subsequent clinical remissions. These patients were not on a low fat diet when the chromatograms before and during the exacerbations were carried out. During the period of remission, however, they were given the low fat diet. An example of the changes in the chromatograms in one patient is shown in Fig. 3, 4, and 5. The chromatogram prior to exacerbation (Fig. 3) was essentially normal. Five to 6 days after the onset of the exacerbation, and while the neurological signs were still increasing, the chromatogram (Fig. 4) showed an absence both of a large number of central fractions, and of some of the upper fractions (compare with Fig. 1-2). Eight days later the protein pattern was little altered. During clinical remission, and 11 weeks (Fig. 5) later, the chromatogram returned towards normal.

Another example of the protein pattern changes during an exacerbation is shown in Fig. 6-8. It will be noted that many of the upper fractions are absent during exacerbation

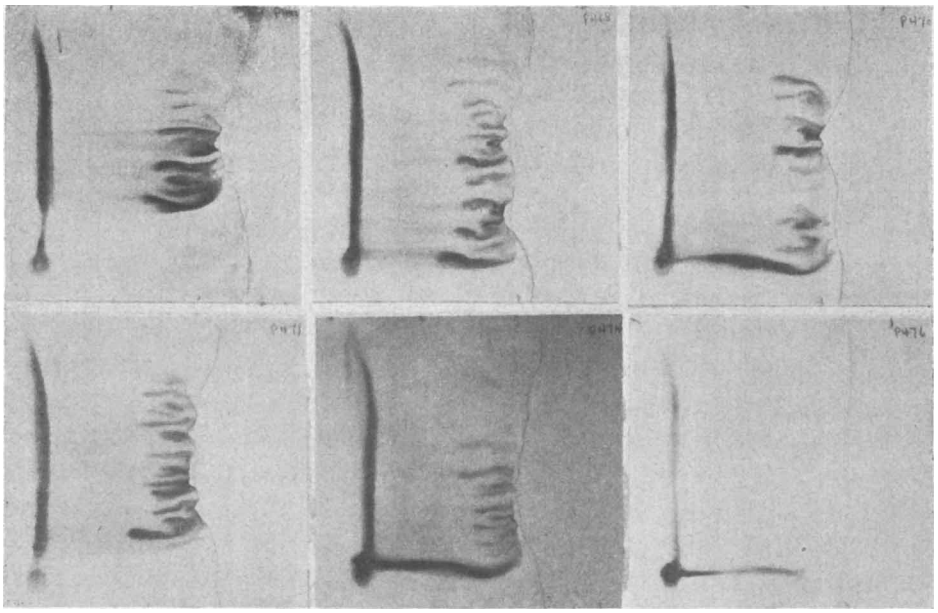

FIG. 3.

Fig. 3, 4, and 5 show a series of chromatograms of a male patient with multiple sclerosis. The chromatogram in Fig. 3 was made from plasma obtained April 6, just before an exacerbation. Fig. 4 shows a chromatogram during the exacerbation (April 13), and Fig. 5 (June 26) shows a chromatogram during remission.

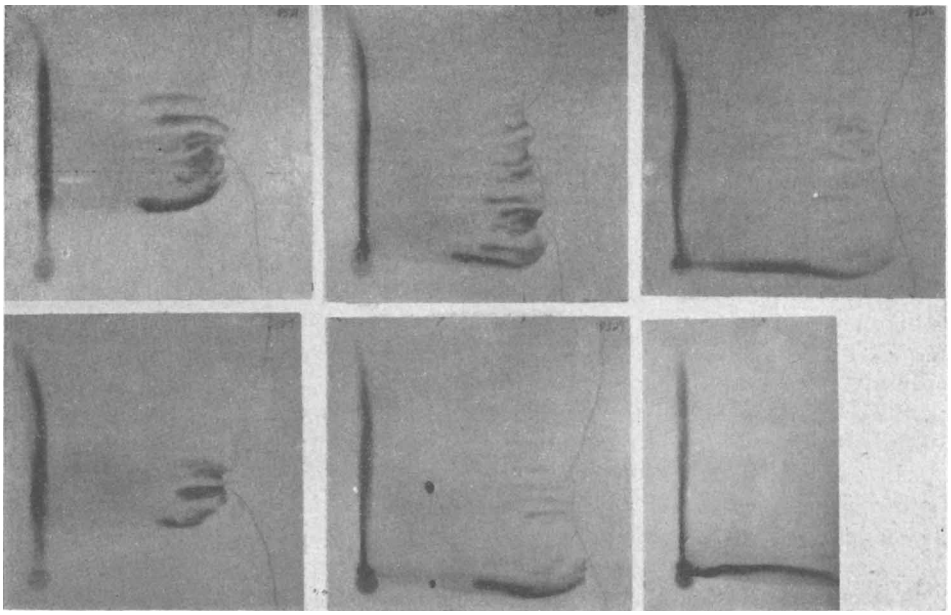

FIG. 4.

(Fig. 7) and that these gradually reappear in the subsequent chromatograms (see Fig. 8; 5 weeks later).

Eleven other patients, without clinical evidence of exacerbation of disease, exhibited changes which were considered distinctly ab-

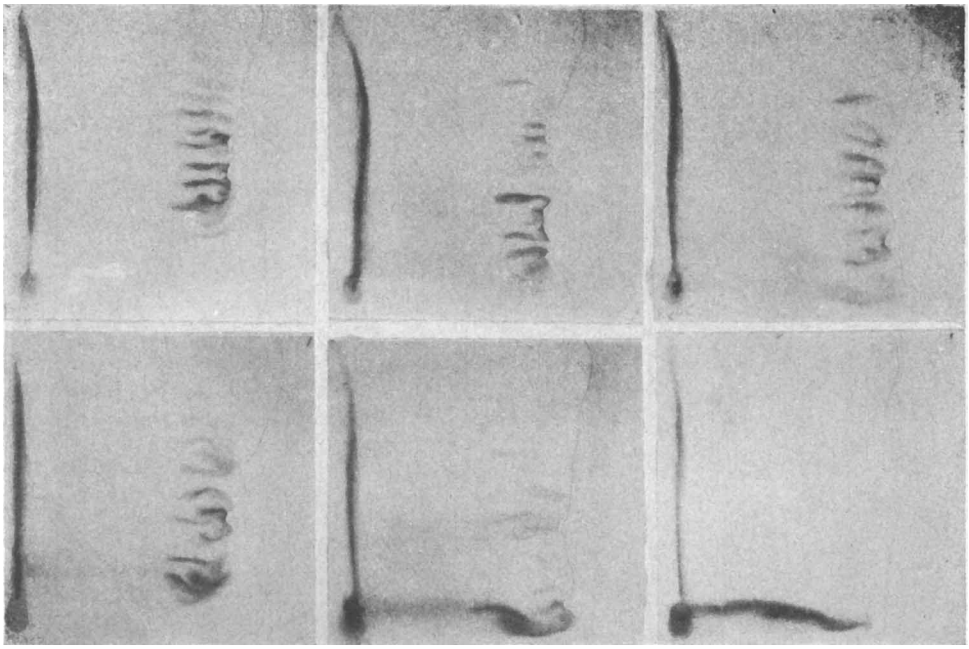

FIG. 5.

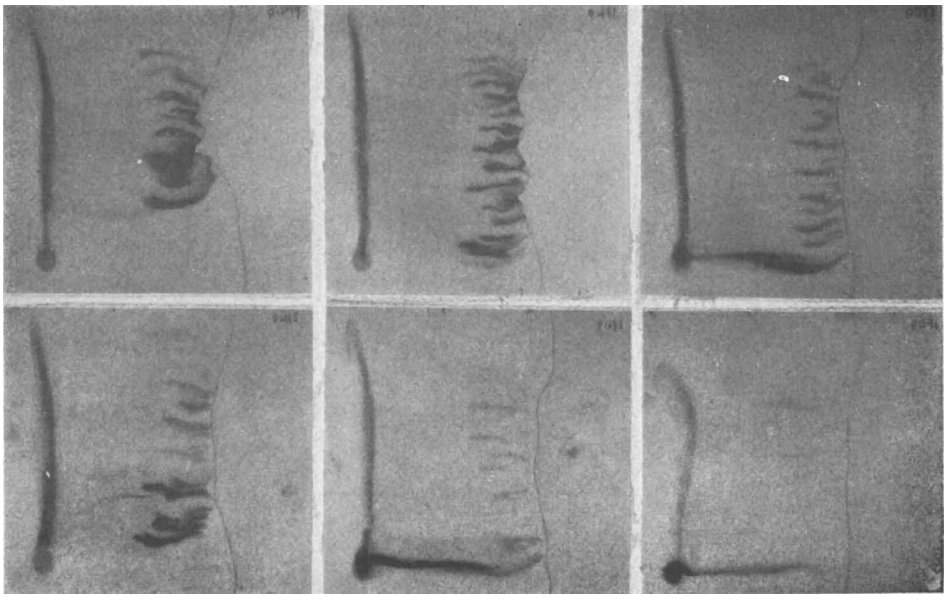

FIG. 6.

Fig. 6, 7, and 8 show a series of chromatograms of a male patient with multiple sclerosis. Fig. 6 was chromatographed (April 6) prior to an exacerbation. Fig. 7 shows the chromatograms 3 days after the onset of an exacerbation (April 14) and Fig. 8 (May 18) shows the chromatogram during remission.

normal, in one or more chromatograms. All of these patients suffered from a relatively active disease as judged by the occurrence of frequent and severe exacerbations during the preceding years. Three additional patients with steadily progressive disability consisting

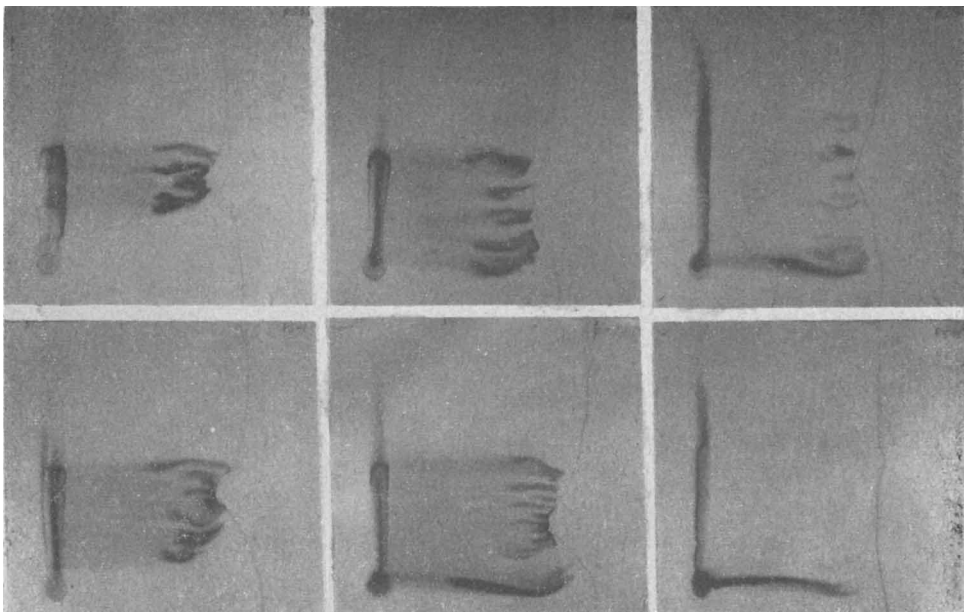

FIG. 7.

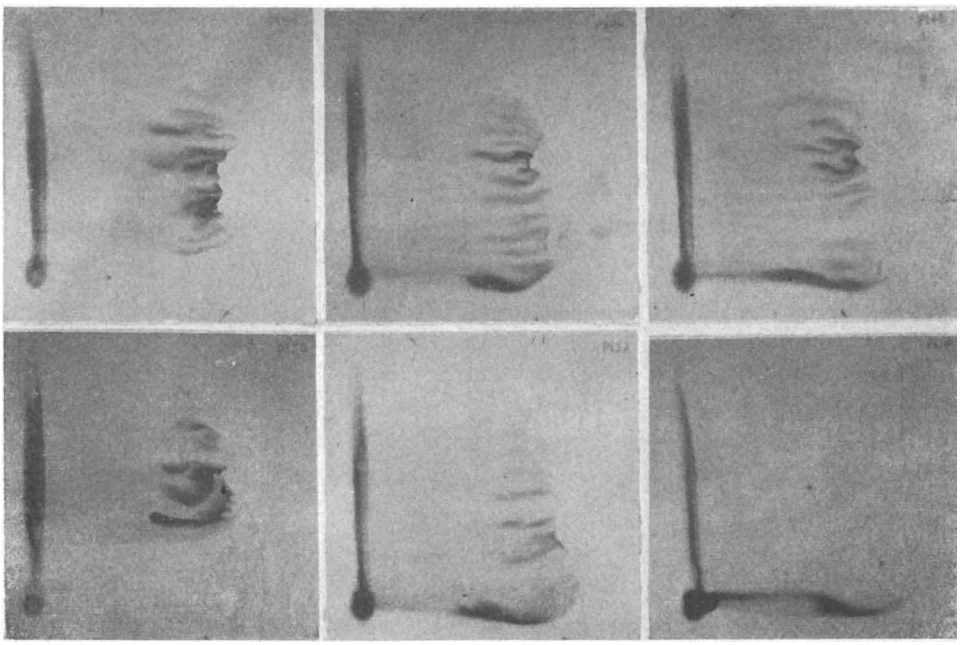

FIG. 8.

of mixed ataxia and spasticity displayed definite alterations of the protein pattern in the chromatograms (see *e.g.* Fig. 9).

Effects of blood transfusions. Preliminary experiments have indicated that, following transfusion, the protein chromatogram tends to revert to normal. This matter is being investigated.

Discussion. The significance of the changes which have been observed in the

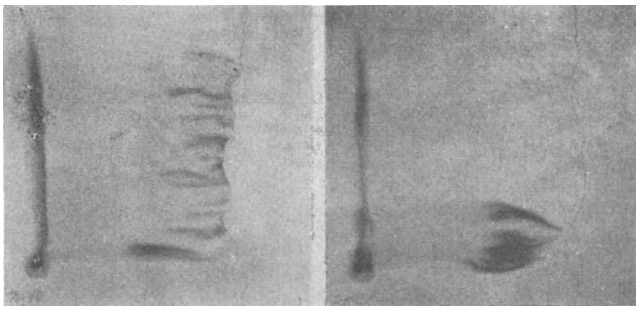

FIG. 9.
Chromatogram of a patient with steadily progressive symptoms of multiple sclerosis. Only the 0.02 ml Tween 81 (left hand side) and 0.01 Tween 85 (right hand side) conditions were used.

plasma proteins of patients with multiple sclerosis is not known. The limited observations suggest that these changes usually do not precede a clinical exacerbation of the disease by an appreciable interval of time. It is difficult to assess the time interval, however, because the exact time of the onset of an exacerbation cannot be established with certainty. Neither do the changes in the protein pattern continue to be present for many weeks after the onset of the exacerbation. If the pattern of plasma proteins shown by paper chromatography is secondary to, and determined by the amount of nervous tissue undergoing change, then it is reasonable to assume that small lesions (for example mild retrobulbar neuritis) might not alter the protein pattern. On the other hand, extensive lesions might occur in so-called silent areas of the brain and produce marked changes in the chromatograms, and yet be unaccompanied by symptoms.

The chromatograms may reflect changes in the blood which occur concomitantly with, or even prior to, the development of the lesions in the nervous system. It may also be considered that the proteins might be modified by the chemical changes attending the occurrence of these lesions.

The abnormalities in the plasma protein chromatograms do not appear to be specific for multiple sclerosis since very abnormal patterns have also been observed in multiple myeloma and liver cirrhosis.¶

Unpublished observations by one of us (R.L.S.) have shown that sedimentation rates of patients with multiple sclerosis (determined at weekly or monthly intervals) are frequently abnormally high, but the evidence so far has not established a clear correlation between such changed sedimentation rates and the abnormal chromatograms shown by these patients. No attempt was made to correlate the plasma protein patterns with the Lange reaction in the spinal fluid.

Summary. Using the paper chromatographic technic significant changes in the protein patterns have been demonstrated in patients with multiple sclerosis. We are unable to state at present the relationship of these changes to the activity of the disease, but our evidence would seem to indicate that they may occur concomitantly with, or immediately following the onset of exacerbation. Furthermore the changes in protein patterns revert to normal during remissions.

¶ Unpublished observations by Franklin and Quastel.

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