Serum lipoproteins in multiple sclerosis determined by cellulose acetate electrophoresis¹

K. GAVRILESCU, R. S. MELLICK², AND W. H. McMENEMEY

Maida Vale Hospital for Nervous Diseases, London

In previous papers (Gavrilescu, Jellinek, and McMenemey, 1961, Gavrilescu and McMenemey, 1964) on comparative studies on the protein fractions in the serum and cerebrospinal fluid in multiple sclerosis we have noted increases in the α_2 and β globulins in the serum. Although not specific for this disease these changes are of interest, particularly as multiple sclerosis is considered to be purely a disease of the central nervous system unassociated with primary systemic disturbances or manifestations. As the greatest proportion of the lipoproteins on cellulose acetate migrate at a similar rate to the α_2 globulins we thought it important to investigate the possibility of an increase in the lipoproteins influencing the protein pattern, and in particular, the globulin changes mentioned above.

MATERIAL

Our material consists of 43 cases of multiple sclerosis. Of these 28 had what was considered, on clinical grounds, to be active disease, while 15 had chronic disease without evidence of any change over the period of observation. Their ages varied between 18 and 68 years. The sex distribution was approximately equal. Our normal controls consisted of 27 healthy adults taken from a slightly younger age group (20-40) with an approximately equal sex distribution.

Serum was collected in the fasting state from the controls and from 19 of the multiple sclerosis cases. The other 24 sera were post-absorptive, blood having been taken from patients soon after a light breakfast or at different hours of the afternoon. Most specimens were examined within a few days; however, it was found that refrigeration at 4°C. for as long as two months did not produce significant changes quantitatively, although some slight alteration in the migration rates were noted. It was found that there was no significant difference in the lipoprotein pattern between post-absorptive and fasting sera.

METHOD

The electrophoretic separation on cellulose acetate was performed in the usual manner (Kohn, 1958) using a veronal buffer pH 8.6, ionic strength 0.05. The strips were 5 × 10 cm. We applied 10 μ l. of serum to the strips. After running for two hours in the electrophoretic tank the strips were dried and cut along the middle. Half were stained with Ponceau S for proteins and the other half stained for lipoproteins, using Kohn's (1961) method based on Schiff's stain after preliminary ozonization. We performed this chemically with barium powder introduced in a beaker placed in an ozonizing chamber to which was added concentrated sulphuric acid. The strips were left in this atmosphere for 30 minutes rinsed in N/10,000 HCl and placed in Schiff's stain for two hours. They were then washed three times in a reducing solution, for which potassium metabisulphite crystals in N/10 HCl were used. The final wash was in N HCl. The strips were scanned on the Chromoscan instrument and results expressed as percentages. On several occasions the reproducibility of the technique and results were tested and affirmed.

RESULTS

The lipoproteins on cellulose acetate migrate at rates similar to the albumin and the α_1 and α_2 globulins. However, there are sometimes differences as can be seen in the strips photographed and reproduced here. The α_1 lipoprotein has a smaller molecular weight than the α_1 globulin and this permits it sometimes to migrate more quickly than the α_1 globulin and indeed sometimes to overlap the albumin band slightly. It is seen, therefore, sometimes lying between, and overlapping, the α_1 globulin on the one hand and the albumin on the other. The lipoproteins migrating in the albumin band occasionally form a sort of compressed ring with fast and slow components. We have called these lipoproteins the fast and slow albumins.

NORMAL CONTROLS Of the 27 cases we recognized three separate fractions in 16 cases, two fractions in nine cases, and four fractions in two cases. All these fractions would be readily scanned. In several strips

¹Presented at the 5th International Congress of Clinical Pathology, Mexico City, 1963.

²Present address Guy's Hospital Medical School, London, S.E.1.

FIG. 1. Three-fraction lipoprotein profile from a normal control.

FIG. 2. Three-fraction lipoprotein profile from a patient with multiple sclerosis.

FIG. 3. Three-fraction lipoprotein profile from a patient with multiple sclerosis showing a normal α_2 lipoprotein in the presence of an increased α_2 globulin.FIG. 4. Four-fraction lipoprotein profile showing a slow and fast albumin and a lipoprotein lying between the slow albumin and the α_1 globulin.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

Normal 1 $\alpha_2 = 56.5\%$ $\alpha_1 = 30.0\%$

Slow albumin = 13.5%

 $\alpha_2 = 53.4\%$ $\alpha_1 = 29.2\%$

Slow albumin = 17.4%

 $\alpha_2 = 57.0\%$ $\alpha_1 = 27.1\%$

Slow albumin = 15.9%

 $\alpha_2 = 50.3\%$ $\alpha_1 = 30.8\%$

Slow albumin = 16.6%

Fast albumin = 2.3%

there were traces of lipoprotein visible with the naked eye throughout the whole protein spectrum, but the amounts were so small that scanning was not possible.

The results (Table I) show clearly that most of the lipoproteins migrate in the α_2 band and also that the α_1 lipoproteins seem to have an inverse relationship with the slow albumins. Two band strips and three band strips are considered separately and for completeness the range of all the lipoprotein fractions we were able to scan are recorded in the third part of the table.

MULTIPLE SCLEROSIS We have considered the whole multiple sclerosis group together (Table II) and the active cases separately (Table III). Of the 28 active cases, 22 had three fractions and six had two fractions. Again the largest proportion of the lipoproteins migrate in the α_2 band and there is an inverse

relationship between the α_1 lipoproteins and the slow albumin. Fast albumin was not seen in the active cases. Because of the small proportion of cases of multiple sclerosis with a fast albumin, and because of its presence in the normal group, no particular significance was attached to its absence from the 28 cases of active disease.

Of the whole multiple sclerosis group there were three fractions in 32 cases, two fractions in seven cases, and four fractions in four cases.

The tables show the proportion of the various lipoproteins present in two-fraction and three-fraction strips, and again, as with the normal group, the third part of Tables II and III shows the overall range for each lipoprotein fraction in the group considered as a whole.

Statistical comparison of the α_1 , α_2 , and slow albumin lipoprotein fractions between the whole multiple sclerosis group, the active multiple sclerosis

TABLE I

LIPOPROTEINS IN NORMAL GROUP

α_2 Lipoprotein (%)	α_1 Lipoprotein (%)	'Slow' Albumin (%)	'Fast' Albumin (%)
<i>Three-fraction strips (16 cases)</i>			
Range	49.2—61.1	15.6—32.1	10.3—26.0
Mean	55.6	26.0	17.4
S.D.	4.1	4.9	4.3
<i>Two-fraction strips (9 cases)</i>			
α_2	α_1		
Range	51.3—67.4	32.6—48.7	
Mean	58.5	41.5	
S.D.	5.2	5.2	
<i>Two-, three-, and four-fraction strips (27 cases)</i>			
α_2	α_1		
Range	49.2—67.4	15.6—48.7	10.3—26.0
Mean	56.5	31.2	17.4
S.D.	4.6	8.9	4.3

TABLE II

LIPOPROTEINS IN ALL CASES OF MULTIPLE SCLEROSIS

α_2 Lipoprotein (%)	α_1 Lipoprotein (%)	'Slow' Albumin (%)	'Fast' Albumin (%)
<i>Three-fractions (32 cases)</i>			
Range	46.9—70	8.5—32.2	11.0—21.5
Mean	56.7	26.1	16.3
S.D.	3.9	4.4	3.0
<i>Two fractions (7 cases)</i>			
Range	56.0—79.5	20.5—44.0	
Mean	64.6	35.4	
S.D.	8.3	8.3	
<i>Whole group (43 cases)</i>			
Range	46.9—79.5	8.5—44.0	11.0—21.5
Mean	58.0	27.5	16.3
S.D.	6.3	6.2	3.0

TABLE III

LIPOPROTEINS IN CLINICALLY ACTIVE CASES OF MULTIPLE SCLEROSIS

α_2 Lipoprotein (%)	α_1 Lipoprotein (%)	'Slow' Albumin (%)
<i>Three fractions (22 cases)</i>		
Range	46.9—70	8.5—32.2
Mean	57.8	25.4
S.D.	4.6	5.3
<i>Two fractions (6 cases)</i>		
Range	56.0—79.5	20.5—44
Mean	64.6	35.4
S.D.	8.3	8.3

group, and the normal group using Student's *t* test is shown in Table IV. There is no significant increase in any of the fractions, although there appears to be a significant decrease in the α_1 lipoproteins in the multiple sclerosis group considered as a whole. This is not evident if the active cases only are considered.

We (Gavrilescu *et al.*, 1961; Gavrilescu and McMenemy, 1964) and others (Durrum, Paul and Smith, 1952; Swahn, 1952) have previously reported an increase in the α_2 globulin in the serum in multiple sclerosis. This finding was confirmed in the present series and α_2 globulin increase above the upper limit of normal (0.9 g.%) was noted in 57% of the cases.

TABLE IV

COMPARISON OF MULTIPLE SCLEROSIS GROUP WITH NORMALS

Lipoproteins		
<i>Three fractions</i>		
α_2 (MS) compared with α_2 (N)	$P < 0.4$	> 0.3
α_1 (MS) compared with α_1 (N)	$P < 0.5$	> 0.4
'Slow' albumin (MS) compared with slow albumin (N)	$P < 0.4$	> 0.3
<i>Two fractions</i>		
α_2 (MS) compared with α_2 (N)	$P < 0.2$	> 0.1
α_1 (MS) compared with α_1 (N)	$P < 0.2$	> 0.1
<i>Total group</i>		
α_2 (MS) compared with α_2 (N)	$P < 0.3$	> 0.2
α_1 (MS) compared with α_1 (N)	$P < 0.05$	> 0.02
'Slow' albumin (MS) compared with slow albumin (N)	$P < 0.4$	> 0.3
$P < 0.05$ is considered significant		

TABLE V

COMPARISON OF ACTIVE MULTIPLE SCLEROSIS CASES WITH NORMALS

Lipoproteins		
<i>Three fractions</i>		
α_2 (MS) compared with α_2 (N)	$P < 0.4$	> 0.3
α_1 (MS) compared with α_1 (N)	$P < 0.5$	> 0.4
'Slow' albumin compared with 'slow' albumin (N)	$P < 0.4$	> 0.3
<i>Two fractions</i>		
α_2 (MS) compared with α_2 (N)	$P < 0.2$	> 0.1
α_1 (MS) compared with α_1 (N)	$P < 0.2$	> 0.1
<i>Whole group</i>		
α_2 (MS) compared with α_2 (N)	$P < 0.1$	> 0.05
α_1 (MS) compared with α_1 (N)	$P < 0.7$	> 0.6
'Slow' albumin (MS) compared with 'slow' albumin (N)	$P < 0.3$	> 0.2
$P < 0.05$ is considered significant		

DISCUSSION

Macheboeuf in 1929 was the first to mention lipoproteins, which he considered as 'cenapses' of linked cholesterol, lecithin, or fatty substance to the protein molecule.

Much later in 1953 with the work of Durrum *et al.* (1952), based on paper electrophoresis, wider possibilities were opened for their study. The initial staining techniques, promoted by Kunkel and Slater (1952) Rosenberg (1952), Swahn (1952), and others, experimenting with Sudan black B, Sudan IV, Sudan III, and oil red O, were not very satisfactory on paper strips. Because of this we decided three years ago to abandon the use of paper and began the study afresh using cellulose acetate following Kohn's method.

Multiple sclerosis is not known to have a somatic component outside the central nervous system. For this reason the finding of an elevated α_2 globulin in 57% of cases after due care was taken to exclude those with complicating inflammatory conditions such as urinary infection, is of interest. The work above has shown that the elevation in the α_2 globulins is

not due to, or related to, an increase in the α_2 lipoproteins.

Zilkha and McArdle (1963), using a chromatographic technique, have produced strong evidence that breakdown products of myelin are detectable in phospholipid increases in cerebrospinal fluid. Roboz, Hess, Forster, and Temple in 1954 postulated that if the breakdown products of myelin found their way into the serum they would be expected to act as neutral fat. They investigated the serum lipoproteins in multiple sclerosis, using paper electrophoresis but were unable to detect any increase in the neutral fat. Even if their postulate were correct, because of the great dilution factor involved one would not expect to find any changes by electrophoretic methods. We, too, using a more sensitive technique, have been unable to detect any increase in the lipoproteins in the serum.

SUMMARY

The serum lipoproteins, using cellulose acetate electrophoresis, were investigated in 27 normal controls and in 43 cases of multiple sclerosis. There was no significant increase in the lipoproteins.

There was a slight but significant decrease in the α_1 lipoproteins in the multiple sclerosis group if the

active and chronic cases were considered together. There was no such decrease apparent in the active group alone.

The increase in the α_2 serum globulins reported in previous papers was confirmed in the present study and was found in 57% of cases. This increase does not appear to be related to an increase in the lipoproteins.

We express our thanks to Mr. G. Cox for the photography and to Miss A. M. Garbis for help with the calculations. One of us (K.G.) is in receipt of a fellowship from the Multiple Sclerosis Society of the United Kingdom.

REFERENCES

- Durrum, E. L., Paul, M. H., and Smith, E. R. B. (1952). *Science*, **116**, 428.
 Gavrilescu, K., Jellinek, E. H., and McMenemey, W. H. (1961). *Wld Neurol.*, **2**, 709.
 —, and McMenemey, W. H. (1964). *Suppl., Z. Immun. Forsch.*, in the press.
 Kohn, J. (1958). *Nature (Lond.)*, **181**, 839.
 — (1961). *Ibid.*, **189**, 312.
 Kunkel, H. G., and Slater, R. J. (1952). *J. clin. Invest.*, **31**, 677.
 Macheboeuf, M. A. (1929). *Bull. Soc. Chim. biol.* **11**, 268 and 485.
 Roboz, E., Hess, W. C., Forster, F. M., and Temple, D. M. (1954). *A.M.A. Arch. Neurol. Psychiat.*, **72**, 154.
 Rosenberg, I. N. (1952). *Proc. Soc. exp. Biol. (N.Y.)*, **80**, 751.
 Swahn, B. (1952). *Scand. J. Clin. lab. Invest.*, **4**, 98.
 Zilkha, K. J., and McArdle, B. (1963). *Quart. J. Med.*, **32**, 79.