Lipid studies in the blood and brain in multiple sclerosis and motor neurone disease

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SYNOPSIS The lipid patterns of plasma, red blood cells, and leucocytes from normal controls and from patients with multiple sclerosis and motor neurone disease have been studied by thin-layer chromatography. The fatty acid composition of cholesterol esters and lecithin in plasma from normal subjects and from patients with multiple sclerosis are reported. The fatty acid composition of lecithin of cerebral white matter from normal control subjects, from multiple sclerosis, and from motor neurone disease as well as of cholesterol esters in multiple sclerosis are recorded and compared. No significant abnormalities were found in the blood lipid profile in multiple sclerosis, but in motor neurone disease red cell sphingomyelin was slightly reduced and white cell lecithin slightly increased. The fatty acids of plasma cholesterol esters showed a slight decrease in palmitic and stearic acid in multiple sclerosis. The fatty acids in lecithin in multiple sclerosis, both in the apparently normal white matter and in the plaques, showed a slightly increased degree of saturation, while the loss of unsaturated acids was in oleic acids.

The lipid composition of brain, blood plasma, and cerebrospinal fluid in multiple sclerosis has attracted the attention of many workers in recent years. A rise in the cephalin level in the cerebrospinal fluid has been recorded (McArdle and Zilkha, 1962; Zilkha and McArdle, 1963) in the more acute stages of the disease, although other workers (Phillips and Robinson, 1963) did not obtain such a marked degree of abnormality. The lipid composition of the apparently normal white matter was shown to be abnormal in multiple sclerosis (Cumings, 1953), while Baker, Thompson, and Zilkha (1963) showed abnormalities in fatty acid composition in similar areas of the brain. They recorded that there was a greater amount of saturated as compared with unsaturated fatty acid in brain lecithin than in the white matter of normal subjects. The relationship of this change to the aetiology of multiple sclerosis is as yet unknown, but it could represent the initial change in an altered myelin structure.

This paper records our findings in a study of the lipids in plasma, red blood cells, and white blood cells in normal subjects, in patients with multiple sclerosis and with motor neurone disease. The fatty acid composition of lecithin and of esterified

cholesterol in cerebral white matter and in plasma has also been made in the same diseases.

MATERIAL AND METHODS

Blood was obtained from 14 patients with multiple sclerosis, from five with motor neurone disease, and from 11 control subjects who were not suffering from organic neurological disease. Plasma was obtained after centrifugation of citrated blood (1 ml. of 3·8% sodium citrate to 9 ml. blood), and the red cells were obtained free from plasma. For the white blood cells the method of Lalezari (1961) using polybrene was employed.

Cerebral white matter from the frontal or parietal lobes was obtained from five normal controls, from four cases of multiple sclerosis, and from two of motor neurone disease at necropsy. Both apparently normal white matter as well as plaque material was obtained from the brains of the cases of multiple sclerosis.

The lipids from both blood and brain were extracted by the method of Folch, Lees, and Sloane Stanley (1957) and estimations of the total lipid phosphorus were performed using the Bartlett procedure as given by McArdle and Zilkha (1962), except that 1-amino-2 napthol-4 sulphonic acid (Eastman Kodak Company) was used in the final stages. The amount of lipid extract used was such as would contain between 0·1 μg. and 1·0 μg. phosphorus in each determination.

The lipid extracts from plasma, red blood cells, and

white cells were examined by thin-layer chromatography (Müldner, Wherrett, and Cumings, 1962), the phosphorus-containing compounds visualized by the use of 50% sulphuric acid, and the various fractions cut out and the phosphorus in each fraction estimated. Control runs of the same lipid extracts on the same plates were stained by the molybdate-perchloric acid reagent (Wagner, Hörhammer, and Wolff, 1961) to demonstrate the exact site and presence of a phosphorus-containing compound.

Lecithin and cholesterol esters in plasma and brain were prepared for examination for their fatty acid composition by the following techniques. The lecithin fraction, visualized as previously, was removed from each plate after preparative thin-layer chromatography, performed as for the phosphorus estimations but with a few modifications. The lipid extract was applied to the plate under a stream of nitrogen and the chromatography tank was filled with nitrogen before use. Cholesterol esters were prepared by the method of Brown and Johnston (1962) but with the use of n-heptane for n-hexane and without incorporating 2′,7′dichlorofluorescin in the gel. The samples were spotted on the plates and run in a manner similar to those for lecithin. The cholesterol esters were located by spraying with 2′, 7′ dichlorofluorescin, and by examining and removing them under ultraviolet light.

The methyl esters were prepared by the method of Stoffel, Chu, and Ahrens (1959), the transesterification step being performed in the presence of silicic acid but with microsublimation omitted. The analysis of the methyl esters was done by gas liquid chromatography using a Pye Panchromatogram at 180°, on a column of 20% polyethylene glycoladipate on celite and with a macro Argon ionization detector.

RESULTS

BLOOD Thin-layer chromatography of the lipids of plasma, red cells, and white cells showed four major components in all specimens similar to the findings of Robinson and Phillips (1963). These last workers claimed that they corresponded to lysolecithin, sphingomyelin, lecithin, and ethanolamine phosphatide. McArdle and Zilkha (1962), using silicic-acid-impregnated paper chromatography, included ino-sitol phosphatide with lysolecithin and serine phosphatide with the ethanolamine phosphatide. As shown by Müldner *et al.* (1962) thin-layer chromatographic separation as used here resulted in serine phosphatide being close to choline lyso-phosphatide, and with ethanolamine lysophospha-tide near to choline phosphatide. However, for convenience the names given by Robinson and Phillips (1963) will be used although it is realized that two of the named compounds may not be present in an absolutely pure state.

The mean results obtained from the blood samples in all three diseases are recorded in Table I. The normal plasma total phospholipid figures differ little from those of either McArdle and Zilkha (1962) or Robinson and Phillips (1963) while the red cell totals hardly differ from the figures of Reed, Swisher, Marinetti, and Eden (1960). There are some small differences in the individual fractions, mainly in the plasma sphingomyelin and lecithin as compared with results of other workers in that their lecithin totals were slightly higher than those recorded here.

All the figures were submitted to statistical analysis and no significance was found for the total phospholipid or for any of the fractions in multiple sclerosis as compared with the normals. The results for sphingomyelin in the red cells in motor neurone disease, using Student's t test, gave a value of $P < 0.05$ while in the white cell lecithin a figure of $P < 0.02$ was calculated. None of the values for any of the other fractions in motor neurone disease was significant.

Table II shows the fatty acid analyses of plasma cholesterol esters in normal controls and in multiple sclerosis. The only differences between the patterns of fatty acids in multiple sclerosis as compared with the normal are in palmitic acid (16 : 0) and stearic acid (18 : 0), these saturated acids being decreased in multiple sclerosis giving figures of $P < 0.05$ and 0.02 respectively. There were no significant differences in any of the unsaturated acids, including

TABLE I

PHOSPHOLIPIDS IN PLASMA, RED BLOOD CORPUSCLES (R.B.C.), AND WHITE BLOOD CORPUSCLES (W.B.C.)

	Total Phospholipid[1] Mean ± S.D.	Lysolecithin (%) Mean ± S.D.	Sphingomyelin (%) Mean ± S.D.	Lecithin (%) Mean ± S.D.	Ethanolamine Phosphatide (%) Mean ± S.D.
Plasma, control	9·52 ± 1·97	14·05 ± 9·07	23·63 ± 3·09	54·3 ± 12·4	8·02 ± 6·71
Plasma, motor neurone disease	8·92 ± 2·7	17·44 ± 6·0	20·12 ± 4·1	54·74 ± 4·97	7·7 ± 5·1
Plasma, multiple sclerosis	8·84 ± 1·31	17·56 ± 3·04	25·66 ± 7·58	48·59 ± 10·2	7·64 ± 3·94
R.B.C., control	13·37 ± 1·8	7·27 ± 4·34	32·97 ± 2·05	36·1 ± 16·9	23·33 ± 10·5
R.B.C., motor neurone disease	12·7 ± 2·42	9·66 ± 4·92	28·38 ± 3·33	29·86 ± 4·4	30·28 ± 5·28
R.B.C., multiple sclerosis	13·67 ± 2·05	6·57 ± 4·4	31·85 ± 4·79	31·24 ± 3·35	29·5 ± 5·5
W.B.C., control	471·0 ± 187·9	11·13 ± 6·3	24·31 ± 3·54	38·23 ± 2·49	26·27 ± 5·88
W.B.C., motor neurone disease	521·2 ± 334·9	9·08 ± 6·5	20·5 ± 5·01	42·28 ± 1·9	24·44 ± 6·23
W.B.C., multiple sclerosis	511·0 ± 255·8	10·68 ± 4·79	21·46 ± 6·43	39·26 ± 4·89	28·29 ± 5·08

[1]Results for plasma in mg./100 ml., for R.B.C. in mg./100 ml. packed red cells, for W.B.C. in mg./100 ml. blood.

TABLE II

FATTY ACID ANALYSIS OF PLASMA CHOLESTEROL ESTERS[1]

Fatty Acids	Normal Mean ± S.D.	Multiple Sclerosis Mean ± S.D.
Saturated		
16:0	17·18 ± 1·94	14·93 ± 2·50
18:0	2·34 ± 0·58	1·67 ± 0·63
Total[2]	23·05 ± 2·63	19·63 ± 3·44
Unsaturated		
16:1	7·11 ± 2·46	7·41 ± 1·82
18:1	24·9 ± 3·18	24·14 ± 2·51
18:2	36·9 ± 4·83	38·75 ± 5·76
20:4	4·81 ± 0·89	5·21 ± 0·38
Total	77·32 ± 2·05	79·13 ± 2·08

[1]Values are expressed as percentage of total fatty acid methyl esters.
[2]Traces of other acids were present in total.

linoleic acid. A review of the case material showed that the clinically more acute and the more advanced cases of multiple sclerosis did not show any significant biochemical differences as compared with the remainder of the patients in this group.

Table III shows the fatty acid analysis of plasma lecithin and no statistically significant abnormalities were found.

TABLE III

FATTY ACIDS IN LECITHINS IN PLASMA[1]

Fatty Acids	Normal Mean ± S.D.	Multiple Sclerosis Mean ± S.D.
Saturated		
14:0	1·65 ± 0·68	1·57 ± 0·64
16:0	32·95 ± 2·45	33·88 ± 4·04
18:0	12·70 ± 1·13	13·86 ± 3·21
Total[2]	48·59 ± 2·10	50·19 ± 3·92
Unsaturated		
16:1	1·82 ± 1·72	2·32 ± 1·23
18:1	16·99 ± 2·44	16·61 ± 2·40
18:2	19·40 ± 3·85	18·67 ± 2·46
20:3	2·40 ± 0·62	2·28 ± 0·73
20:4	8·34 ± 1·37	8·06 ± 1·67
Total	50·63 ± 2·87	49·59 ± 3·82

[1]Values are expressed as percentage of total fatty acid methyl esters
[2]Traces of other acids were present in total.

BRAIN The fatty acid composition of lecithin in the white matter of normal controls and in motor neurone disease, and of apparently normal white matter in multiple sclerosis, and in the plaques in multiple sclerosis is given in Table IV. There is a slight increase in saturated and a slight decrease in unsaturated acids in the apparently normal white matter in multiple sclerosis (in both $P < 0.1$), with the greater change ($P < 0.01$) in oleic acid (18 : 1), while in the plaques the same changes are evident with the figure for oleic acid (18 : 1) being $P < 0.05$, when compared with normal white matter.

The figures for the fatty acids in cholesterol esters in the plaques in multiple sclerosis are included in Table IV. Comparison with cholesterol esters from a normal brain is not possible as these are not found or are only present in trace amounts, but there is a very considerable difference from the plasma cholesterol ester fatty acid composition.

DISCUSSION

It was hoped that some abnormality would have been found in the lipid composition of the blood in multiple sclerosis and in motor neurone disease for both these diseases produce severe damage to nervous tissue, but the results have not demonstrated any significant variation from the normal, apart from one or two minor aspects. It has already been stated that some changes have been reported in the cerebro-spinal fluid lipid profile in multiple sclerosis, but this is not surprising in any disease in which gross cerebral destruction takes place.

The technique used here for normal red cell lipids has yielded results very comparable with similar studies by Reed et al. (1960), although the more recent method of Ways and Hanahan (1964), which is now in use, should provide a more exact

TABLE IV

FATTY ACID COMPOSITION OF LECITHIN AND CHOLESTEROL ESTER IN WHITE MATTER[1]

Fatty Acids	Lecithin				Cholesterol Ester
	Normal Mean ± S.D.	Multiple Sclerosis Apparently Normal Mean ± S.D.	Multiple Sclerosis Plaque Mean ± S.D.	Motor Neurone Disease Mean	Multiple Sclerosis Plaque Mean
Saturated					
14 : 0	4·66 ± 1·18	2·05 ± 1·25	3·40	2·80	5·37
16 : 0	27·14 ± 2·74	27·57 ± 2·67	31·13 ± 4·48	26·70	30·0
18 : 0	9·40 ± 2·71	12·15 ± 1·57	9·53 ± 1·57	11·20	4·45
Total[2]	42·68 ± 1·34	45·70 ± 3·26	46·36 ± 3·83	41·75	44·25
Unsaturated					
16 : 1	5·08 ± 2·57	4·47 ± 3·18	5·66 ± 3·49	4·70	17·45
18 : 1	45·32 ± 2·36	38·35 ± 1·75	40·06 ± 1·81	45·50	27·15
20 : 1	1·88 ± 1·10	1·57 ± 0·88	1·07	2·55	2·52
20 : 4	1·96 ± 0·58	2·20 ± 0·98	2·70	2·15	2·20
Total	56·12 ± 1·60	52·20 ± 3·98	52·60 ± 5·31	57·65	54·12

[1]Values are expressed as percentage of total fatty acid methyl esters.
[2]Traces of other acids were present in total.

TABLE V

FATTY ACIDS IN LECITHIN IN CEREBRAL WHITE MATTER (NORMAL)[1]

Author	*Saturated*			*Unsaturated*			
	16 : 0	*18 : 0*	*Total*	*16 : 1*	*18 : 1*	*20 : 4*	*Total*
Baker *et al.* (1960)	24·6	13·3	37·9	4·9	51·1	3·3	59·3
Bernhard and Lesch (1963)	33·3	10·5	44·7	2·4	52·0		55·3
O'Brien *et al.* (1964)	34·3	13·4	47·7	1·0	45·2	1·3	49·0
Present results	27·1	9·4	42·7	5·1	45·3	2·0	56·1

[1]Values are expressed as percentage of total fatty acid methyl esters.

appreciation of the facts and render results and comparisons more acceptable. The method of white cell separation yields large numbers of such cells, even though contaminated by a few red cells, but the figures for total lipid vary too widely for any reasonable comparisons to be made between normal and disease states. However the relative proportions of the four major constituents show considerably less variation and yet do not show significant differences between the controls and either multiple sclerosis or motor neurone disease.

The fatty acid composition of both lecithin and cholesterol esters in plasma as seen in the normals (Tables II and III) are very similar to those recorded by Hanahan, Watts, and Pappajohn (1960) and by Blomstrand (1960); thus the absence of any marked abnormality found in multiple sclerosis plasma is of importance. It is true that in cholesterol esters there is a slight reduction in total saturated fatty acids but the unsaturated fatty acids are not significantly altered. Indeed linoleic acid (18 : 2), although at slightly higher levels in multiple sclerosis plasma as compared with our value in normals is still lower than the corresponding normal figure of Blomstrand (1960). The values for fatty acids in plasma lecithin obtained by Hanahan *et al.* (1960) are very similar to those reported here, so that the lack of abnormality in multiple sclerosis plasma is worthy of note.

There are a few more reports relating to normal brain lecithin and these are briefly given in Table V. It is seen that there is some degree of variation, for the results obtain by O'Brien, Fillerup, and Mead (1964) demonstrate a rather higher content of saturated and a lower level of unsaturated fatty acids than do those of other workers, while the figures reported here lie midway between those of Baker *et al.* (1963) and those of O'Brien *et al.* (1964), and are very similar to those recently published by Bernhard and Lesch (1963). These last authors found marked differences between different areas of the brain, especially between the cortex and the white matter. These variations render it more difficult to comment on minor abnormalities, but as a similar change was present in both the plaques and the ap-

parently normal white matter in multiple sclerosis, the finding of a slight increase in saturation with a decrease in oleic acid (18 : 1) may be of significance. However, the figures obtained are within the normal range of O'Brien *et al.* (1964) so that caution in interpretation must be observed. No other observations relating to motor neurone disease are available for comparison but the findings reported here for white matter lecithin were not abnormal.

Baker *et al.* (1963), in commenting on their results, state that possible causes of change in the fatty acid composition of lecithin in the apparently normal white matter in multiple sclerosis might be due to a defect in synthesis, a dietary deficiency, or a failure in intestinal absorption. Regarding this latter suggestion, it is of interest that in a small series of patients studied in parallel with a similar control group no abnormality in the standard xylose absorption test using 25 g. doses of xylose has been found. This would be some slight evidence against an abnormality of intestinal absorption in multiple sclerosis.

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