Plasma phospholipids and their fatty acid composition in multiple sclerosis

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Patients with multiple sclerosis, in whom there is evidence of recent clinical deterioration, have been shown to exhibit a reduction both in the proportion and in the absolute level of linoleic acid in the serum (Baker, Thompson, and Zilkha, 1964, 1966). Since the cholesteryl ester fraction of the plasma lipids contains a much higher proportion of linoleic acid than either the phospholipid or triglyceride fractions, the levels of cholesteryl linoleate in serum from normal subjects and from patients with multiple sclerosis have also been investigated (Baker, Sanders, Thompson, and Zilkha, 1965). It was found that the amount of this fraction of the total serum linoleate is strikingly reduced in patients in the active stages of this disease; the reduction in the level of cholesteryl linoleate in the most seriously affected group of patients amounted to 73% of the reduction in total linoleate.

Since it is known that transesterification processes between the cholesterol and the β-linked unsaturated fatty acid of certain of the phospholipids can take place in the serum (Glomset, Parker, Tjaden, and Williams, 1962; Glomset, 1962, 1963), it was decided, in view of these marked changes in the level of cholesteryl linoleate, to investigate also the plasma phospholipids and their fatty acid composition.

The 'neurological controls', i.e., patients suffering from nervous disorders other than primary demyelinating diseases, were also attending the National Hospital, and comprised seven men and 10 women, aged 18 to 66 years.

The measurements that we have made on the plasma phospholipids have been examined statistically, but since no differences between the two sexes became apparent, the men and women have been treated as one group in each case.

Blood was drawn from each subject from the antecubital vein after an overnight fast, and was immediately transferred into tubes containing oxalate (2 mg. potassium oxalate/ml. blood).

The subsequent analyses were carried through 'blind', the analyst being unaware of the identity of the specimen.

**ANALYTICAL METHODS**

**EXTRACTION OF TOTAL LIPIDS** Plasma lipids were extracted by the addition of 19 vol. chloroform-methanol (2:1, v/v) according to the method of Folch, Lees, and Sloane Stanley (1957). The water-washed lower layer was then evaporated to dryness under reduced pressure, and the residue, after repeated evaporation and re-solution in chloroform, was finally dissolved in 50 ml. chloroform. Aliquots of this solution were used for the various determinations carried out.

**ESTIMATION OF TOTAL PHOSPHOLIPIDS** Total phospholipids were determined by estimation of phosphorus in duplicate samples of the total lipid extract by the method of Fiske and Subbarow (1925).

**FATTY ACID COMPOSITION OF TOTAL PHOSPHOLIPIDS** The phospholipids were first separated from the other lipids present in the total lipid extract by thin-layer chromatography on glass plates (20 × 16 cm.) covered by a layer (15 g./plate) of MN-silica gel G8 (Macherey, Nagel & Co.).

Of the washed total lipid extract, 10 ml. was evaporated down to 0-5 ml. (approx.) which was applied to the plate at 15 spots. To another 10 ml. sample, 1 ml. of a standard solution containing 0-22 μ mole of synthetic dipenta-decanoyl lecithin (synthesized by Dr. G. H. de Haas) in chloroform was added, and the whole, after evaporation to 0-5 ml., was applied to a duplicate plate.
The plates were developed in 30% (v/v) ether in hexane, all operations being carried out in a glove box filled with 
N₂ to prevent oxidation.

After developing, the phospholipids were stained with a solution containing 0.005% (w/v) Ponceau Red and
0.2% (w/v) uranyl nitrate in 0.01 N HCl (Hooghwinkel,
Hoogeveen, Lexmond, and Bungenberg de Jong, 1959).

The phospholipids were scraped from the plates after
staining, and directly subjected to methanolysis by heating
in glass-stoppered tubes at 70°C for two hr. under N₂ with
18 ml. methanol (AR) containing 2-6 g. HCl/100 ml.
Then 2 ml. 8N NaOH was added, and the heating con-
tinued for one hour at 70°C.

After cooling and adding 20 ml. H₂O, 10% (v/v)
H₂SO₄ was added until the pH was below 4, and the free
fatty acids were then extracted three times with 25 ml.
amounts of pentane. The extract was dried over an-
hydrous Na₂SO₄, the pentane evaporated, and after the
addition of 1 ml. methanol containing 1:5% (v/v) H₂SO₄ the
mixture was heated for 15 min. at 60°C under N₂ to
convert the fatty acids to their methyl esters (Baker et al.,
1964). The isolation of the methyl esters and their separa-
tion by gas chromatography on 6-ft. polyester columns
were as described by Baker et al. (1964). The area of
each fatty acid 'peak' on the chromatogram, including that
of the methyl pentadecanoate derived from the standard
lecithin, was measured by triangulation; the molar cor-
rection constants for the different fatty acids were
determined as described by Baker et al. (1966).

EXAMINATION OF SEPARATE PHOSPHOLIPID COMPONENTS

The proportions of the separate lecithin, lyssolecithin,
phosphatidyl ethanolamine, and sphingomyelin (together
with lysophosphatidyl ethanolamine) components of
the total phospholipids were determined by thin-layer
chromatography.

Of the lipid extract, 2 ml. evaporated and re-
dissolved in 0-2 ml. chloroform, was applied to thin-
layer plates (20 × 5 cm.) each covered with a layer (3 g./
plate) of silica gel G (E. Merck). After developing in
chloroform:methanol:water (14:6:1, v/v) according to
Müldner, Wherrett, and Cumings (1962), the spots
were stained with iodine vapour. They were then scraped off
the plates and the amount of silica gel present in each spot
was estimated by weighing. The phosphorus present in
each spot was determined in duplicate estimations (with
suitable blanks for the silica gel and for the other re-
gents) by the method of Fiske and Subbarow (1925).

RESULTS

It will be seen from Table I that patients in the in-
active phases of the disease or with evidence of only
slight clinical deterioration during the preceding month
(grade A) showed levels of total phospho-
lipids in the plasma which were indistinguishable
from those of the control group. On the other hand,
in patients exhibiting evidence of moderate or marked
degrees of activity of the disease (grades B and C),
the concentration of total phospholipids in the
plasma was found to be significantly reduced, and
since grades B and C do not differ significantly one
from another they can be combined to give a single
group showing a phospholipid level of 2-63 μmoles/
ml.

TABLE I
CONCENTRATION OF TOTAL PHOSPHOLIPIDS IN THE PLASMA
OF PATIENTS WITH MULTIPLE SCLEROSIS

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>Total Phospholipids (μmoles/ml. ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>17</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td>Grade A</td>
<td>9</td>
</tr>
<tr>
<td>Grade B</td>
<td>10</td>
</tr>
<tr>
<td>Grade C</td>
<td>8</td>
</tr>
<tr>
<td>All grades combined</td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences between means

- Controls v. grade A: not significant
- Controls v. grades B and C: P < 10⁻³

It is therefore not surprising that estimations of
the amounts of the different fatty acids contained
in these phospholipids also show, when expressed

TABLE II
LEVELS OF PHOSPHOLIPID FATTY ACIDS IN THE PLASMA IN MULTIPLE SCLEROSIS (μMOLES/ML. ± S.E.M.)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Controls (17)</th>
<th>Grade A (9)</th>
<th>Grade B (10)</th>
<th>Grade C (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.12 ± 0.01(9)</td>
<td>0.09 (3)</td>
<td>0.12 (4)</td>
<td>0.11 (2)</td>
</tr>
<tr>
<td>16:0</td>
<td>1.95 ± 0.05</td>
<td>2.00 ± 0.06</td>
<td>1.71 ± 0.04</td>
<td>1.55 ± 0.06</td>
</tr>
<tr>
<td>16:1</td>
<td>0.23 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.19 ± 0.03</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>18:0</td>
<td>0.66 ± 0.05</td>
<td>0.62 ± 0.03</td>
<td>0.50 ± 0.02</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>18:1</td>
<td>0.98 ± 0.05</td>
<td>0.88 ± 0.05</td>
<td>0.77 ± 0.05</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>18:2</td>
<td>1.08 ± 0.03</td>
<td>1.16 ± 0.06</td>
<td>1.11 ± 0.07</td>
<td>0.96 ± 0.11</td>
</tr>
<tr>
<td>20:3</td>
<td>0.26 ± 0.03</td>
<td>0.30 ± 0.05</td>
<td>0.20 ± 0.03</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>20:4</td>
<td>0.69 ± 0.05</td>
<td>0.77 ± 0.08</td>
<td>0.64 ± 0.06</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>Others*</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.28</td>
</tr>
<tr>
<td>Total fatty acids (mean of sum of individual fatty acids for each subject)</td>
<td>6.30 ± 0.19</td>
<td>6.40 ± 0.23</td>
<td>5.40 ± 0.06</td>
<td>5.25 ± 0.23</td>
</tr>
</tbody>
</table>

*Nos. in brackets = nos. of subjects.
*Acids have been included in 'others' when present in amounts less than 1% of the total fatty acids.
as μmoles/ml. serum, the same trend, i.e., no significant difference between the control and grade A groups, but evidence of lower levels in grades B and C (Table II).

This trend becomes more apparent if grades B and C are combined and compared with the combined control and grade A groups. Myristic acid (14:0) appears to be an exception, but it must be remembered that this acid is present in amounts so small that it has only been listed separately in a very small number of cases.

When the different fatty acid components of the phospholipids are expressed as percentages of the total fatty acids present in these lipids (Table III) it will be seen that there is little evidence of any striking change in the proportions of any of them as we pass from the control subjects to the patients with multiple sclerosis grouped under the three grades of increasing activity. It would seem fair to conclude that although the total amount of phospholipids in the plasma is reduced in patients in the active stages of the disease, the fatty acid composition of the phospholipids shows no evidence of significant change.

The results of estimations of the separate phospho-

| TABLE III |

PHOSPHOLIPID FATTY ACIDS, EXPRESSED AS MOLAR PERCENTAGES OF THE TOTAL FATTY ACIDS PRESENT IN THESE LIPIDS

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Controls (17)</th>
<th>Multiple Sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade A (9)</td>
<td>Grade B (10)</td>
</tr>
<tr>
<td>14:0</td>
<td>1.9 (9)</td>
<td>1.4 (3)</td>
</tr>
<tr>
<td>16:0</td>
<td>30.9</td>
<td>31.5</td>
</tr>
<tr>
<td>16:1</td>
<td>3.7</td>
<td>3.0</td>
</tr>
<tr>
<td>18:0</td>
<td>10.5</td>
<td>9.8</td>
</tr>
<tr>
<td>18:1</td>
<td>15.5</td>
<td>13.9</td>
</tr>
<tr>
<td>18:2</td>
<td>17.1</td>
<td>18.3</td>
</tr>
<tr>
<td>20:3</td>
<td>4.0</td>
<td>4.7</td>
</tr>
<tr>
<td>20:4</td>
<td>11.0</td>
<td>12.1</td>
</tr>
<tr>
<td>Others</td>
<td>5.4</td>
<td>5.3</td>
</tr>
</tbody>
</table>

lipid components in the plasma are shown in Table IV. The sphingomyelin estimates include the small amount of lysophosphatidyl ethanolamine which is present in plasma, while the trace of phosphatidyl serine is included in the lysolecithin estimates. As is to be expected from the fall in the level of total phospholipids in grades B and C (Table I), the level of lecithin, which is by far the most plentiful of the plasma phospholipids, also shows a reduction in grades B and C. However, when these phospholipid components are expressed as percentages of the total phospholipids (Table IV), it will be seen that no firm conclusion can be drawn about the proportion of each of these phospholipid species owing to the small size of the groups which we have studied.

DISCUSSION

Earlier work on the levels of phospholipids in the blood of patients with multiple sclerosis has resulted in conflicting statements. Thus, Dobin and Switzer (1954) and Persson (1958) have reported raised levels of phospholipids in multiple sclerosis, whereas Chiavacci and Sperry (1952), Bernsohn and Namajuska (1955), and Šercl, Kovarík, and Jicha (1961) reported lowered levels in this disease. Fog (1951), Wilmot and Swank (1952), Jones, Jones, Howard, and Bunch (1954), and Plum and Fog (1959) reported no significant change.

The results given in this paper show a reduction in the total plasma phospholipids in the patients with multiple sclerosis when treated as a single group. However, as we have already emphasized, in a disease such as multiple sclerosis which shows remissions and relapses it is important to separate the results in those cases with active disease (grades B and C) from those with little or no signs of activity. When this is done it will be seen (Table I) that there is no significant difference between the controls and our grade A patients, but that there is a marked reduction in grades B and C compared with the

| TABLE IV |

SEPARATE PHOSPHOLIPID COMPONENTS IN THE PLASMA IN MULTIPLE SCLEROSIS

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Controls (6)</th>
<th>Grade A (6)</th>
<th>Grade B (6)</th>
<th>Grade C (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expressed as μmoles phospholipid component/ml. plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithin</td>
<td>2.10 ± 0.06</td>
<td>1.74 ± 0.05</td>
<td>1.72 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>0.10 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin (with L.P.E.)</td>
<td>0.58 ± 0.05</td>
<td>0.51 ± 0.04</td>
<td>0.48 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Lysolecithin</td>
<td>0.34 ± 0.03</td>
<td>0.38 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Expressed as percentage of total phospholipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lecithin</td>
<td>66.7</td>
<td>64.4</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>3.1</td>
<td>2.6</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin (with L.P.E.)</td>
<td>18.1</td>
<td>18.9</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>Lysolecithin</td>
<td>10.8</td>
<td>14.1</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>L.P.E. = lysophosphatidyl ethanolamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
controls ($P \leq 10^{-4}$). The levels of the separate phospholipid components were analysed in a smaller number of cases; no significant change in the proportions was discernible with these small samples.

When we turn to the findings for the levels of the different fatty acids of the total phospholipid fraction we note a decline in the values for all the fatty acids in grades B and C, commensurate with the fall in the total phospholipid content in the blood of these patients. There is, however, no alteration in the percentage composition of the different fatty acids. This is in contrast to our finding in the case of cholesteryl esters, where not only did the mean absolute level in $\mu$moles/ml. fall progressively with increasing activity of the disease, but the percentage of cholesteryl linoleate in the total cholesteryl ester also fell from a value of 47-5% in the controls to 40-9% in grade C (Baker et al., 1965). In the case of the phospholipid linoleate its proportion remains constant even in the most severely affected patients.

It appears therefore that the lowering in the level of cholesteryl linoleate in patients with recent deterioration is not accompanied by any significant change of phospholipid linoleate. The finding that the total phospholipid content is lowered, again only in those cases of most active disease, means that cholesteryl linoleate is not the only blood lipid to show change in this disease.

SUMMARY

The levels of plasma phospholipids and their fatty acid composition have been determined in specimens from 27 patients with multiple sclerosis and 17 control subjects.

Patients exhibiting the greatest evidence of recent deterioration showed a decline in the level of total phospholipids in the plasma from the 'normal' mean value of 3-17 $\mu$moles/ml. to a value of 2-56 $\mu$moles/ml., whereas patients in remission gave values not significantly different from those of the controls.

The proportions of different fatty acids in the phospholipids were not, however, changed even in patients in the active stages of the disease. Neither did the proportions of different phospholipids show any alteration from the normal.

We would like to thank UNESCO for the award of an IBRO Research Fellowship to one of us (A.M.). Our thanks are also due to Dr. G. H. de Haas, Department of Biochemistry, University of Utrecht, for the gift of a sample of synthetic dipentadecanoyl lecithin, to the physicians in charge of the patients for their helpful cooperation in this work, to the Multiple Sclerosis Society for a grant, and to Mr. K. Kilbourn for his skilled technical help.

REFERENCES


——, Thompson, R. H. S., and Zilkha, K. J. (1964). Serum fatty acids in multiple sclerosis. Ibid., 27, 408-414.


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*J Neurol Neurosurg Psychiatry* 1966 29: 99-102
doi: 10.1136/jnnp.29.2.99

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