Abnormal lipoproteins in multiple myelomatosis

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SUMMARY In a study of the lipoprotein pattern in multiple myelomatosis electrophoresis on agarose gel showed abnormal lipoproteins, named paralipoproteins (p-Lp), in 24 out of 30 normolipidaemic patients. These paralipoproteins were grouped according to their mobility into one or another of the following types: (1) p-Lp1, with a mobility identical with that of γ-globulin, (2) p-Lp2, with a mobility between that of β- and γ-globulin, (3) p-Lp3, with a mobility identical with that of β-globulin. On ultracentrifugation the abnormal lipoproteins were found to have a density above 1.063 g/ml.

Isolated cases of multiple myelomatosis with the appearance of abnormal lipoprotein fractions have been reported (Hollan, 1958; Lennard-Jones, 1960; Neufeld et al., 1964; Koga et al., 1974). In this paper we report the lipoprotein pattern in 30 patients with multiple myelomatosis.

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Table Plasma lipids in 30 patients with multiple myelomatosis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (years) and sex</th>
<th>Class of M-Component</th>
<th>Electrophoresis</th>
<th>Cholesterol (mg/130 ml)*</th>
<th>Triglycerides (mg/100 ml)*</th>
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<td></td>
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*Normal range according to data from our laboratory (Nikiforakis, 1976) for people aged over 40: plasma cholesterol 100-270 mg/100 ml. plasma triglycerides 41-200 mg/100 ml.

Conversion: traditional to SI units—Cholesterol: 1 mg/100 ml ≈ 0.025 mmol/l. Triglyceride: 1 mg/100 ml ≈ 0.012 mmol/l.
Patients and methods

Thirty patients (17 men and 13 women) aged 48 to 88 years suffering from multiple myelomatoses (MM) were studied (Table). All had the following clinical and laboratory findings: osteolytic changes characteristic of MM, anaemia, a sharp peak or narrow band of protein on electrophoresis, myeloma plasma cells in the bone marrow aspirate, and hyper-immunoglobulinaemia (detected by immunoelectrophoresis). Bence Jones proteinuria was present in only three patients (cases 9, 10, 11).

Thirty healthy individuals with normal lipoproteins and of about the same age and sex distribution as the patients served as controls.

Plasma lipids were measured in venous blood collected in tubes containing EDTA (1 mg/ml) after overnight fasting of 12-14 hours.

Triglycerides were measured by the method of Jover (1963) and cholesterol by the method of Abell et al. (1952).

Plasma lipoprotein and serum protein electrophoresis was carried out on fresh samples on agarose gel (Johanson, 1972).

Plasma ultracentrifugation was performed on three selected patients (see below). Lipoproteins were separated by the method of Carlson (1973) into VLDL at a density of 1.006 g/ml and into LDL at a density of 1.063 g/ml. The HDL was obtained from the bottom fraction by the tube-slicing technique after the second centrifugation. After ultracentrifugation the isolated fractions were submitted to electrophoresis on agarose gel.

In three selected patients the abnormal lipid-staining bands isolated by ultracentrifugation (see below) were subjected to electrophoresis on agarose gel. The area corresponded to that stained by Sudan Black. Abnormal material was extracted according to Folch et al. (1957) before staining. The lipid nature of the abnormal bands was confirmed by thin layer chromatography (TLC) on glass plates covered by silica gel (Pyrovolakis et al., 1974). The lipids were developed in a mixture of petroleum ether, diethylether, and acetic acid (82:18:1). Three healthy individuals with normal lipoprotein pattern served as controls.

Results

Quantitative lipid assay

Plasma triglyceride and cholesterol levels were normal in all patients (Table).

Lipoprotein electrophoretic studies

Lipoprotein electrophoresis revealed the presence of abnormal lipid staining bands in 24 out of the 30 patients. These abnormal bands, which we have called paralipoproteins (p-Lp), were grouped according to their mobility into one or another of the following types.

1. In seven patients the paralipoprotein band had a mobility identical with that of $\gamma$-globulin (p-Lp1; Fig. 1a).

2. In eight patients the paralipoprotein band had a mobility identical with that of $\beta$-globulin (p-Lp2; Fig. 1c).

3. In nine patients the paralipoprotein band had a mobility between $\beta$- and $\gamma$-globulin (p-Lp3, Fig. 1b).

The mobility of these abnormal lipoprotein bands corresponded fully to that of the paraprotein in each case (Fig. 2).

![Fig. 1 Electrophoresis on agarose gel of lipoproteins isolated from the plasma of myeloma patients. Note three abnormal lipoprotein fractions, p-Lp1(a), p-Lp2(b), p-Lp3(c), marked by arrows.](http://jcp.bmj.com/)

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Abnormal lipoproteins in multiple myelomatosis after the second ultracentrifugation at a density of 1.063 g/ml (Fig. 2e).

QUALITATIVE LIPID ASSAY
Cholesterol, triglycerides, phospholipids, and free fatty acids were identified by TLC in the three p-Lp bands (p-Lp1, p-Lp2, p-Lp3). No lipids were identified by TLC in the normal lipoprotein pattern in the area corresponding to the p-Lp bands.

LIPOPROTEIN PATTERN AFTER TREATMENT
In two patients with p-Lp1, two with p-Lp2, and three with p-Lp3 the lipoprotein pattern was also studied electrophoretically after two to five courses of treatment by the regimen of Alexanian et al. (1958). The paralipoprotein disappeared completely after the treatment.

ULTRACENTRIFUGATION STUDIES
Further information on the abnormal lipoprotein bands of each type was obtained by ultracentrifugation. The paralipoproteins p-Lp1, p-Lp2, and p-Lp3 were isolated in the bottom fraction with the HDL.

Fig. 2 Electrophoresis of whole plasma (from a myeloma patient) as well as of isolated lipoprotein fractions after ultracentrifugation. (a) Lipoprotein electrophoresis of whole plasma. Note presence of abnormal p-Lp1 fraction (arrow). (b) Lipoprotein electrophoresis of top fraction separated by ultracentrifugation at density of 1.006 g/ml. (c) Lipoprotein electrophoresis of bottom fraction at density of 1.006 g/ml. Note abnormal p-Lp1 fraction (arrow). (d) Lipoprotein electrophoresis of top fraction separated by ultracentrifugation at density of 1.063 g/ml. (e) Lipoprotein electrophoresis of bottom fraction at density of 1.063 g/ml. As illustrated, the p-Lp3 was isolated in the bottom fraction with HDL.

Fig. 3 Lipoprotein electrophoresis before and after treatment of myeloma. Note disappearance of abnormal lipoprotein and paraprotein bands after treatment (e, d).
only in one, a patient of the p-Lp, type. In this case the paraprotein also disappeared completely after treatment (Fig. 3).

Discussion

Sachs et al. (1954), using paper electrophoresis, noted an abnormal lipid staining band which migrated with the abnormal gamma-globulin in five out of 11 patients with multiple myelomatosis. In one with beta-myeloma and another with minor protein abnormalities a lipid band was found between beta- and gamma-globulin. As Sachs doubted that these abnormal lipid staining bands contained true fat he described them as 'lipid-like' and investigated no further. Later Pagé et al. (1974) found a lipid staining post-beta band in patients with multiple myelomatosis while performing a routine lipoprotein electrophoresis on cellulose. He also found this band in 18 out of 20 selected patients with multiple myelomatosis. The nature of these lipid stained bands was not investigated further. Other authors (Hollan, 1958; Lennard-Jones, 1960; Neufeld et al., 1964; Lewis and Page, 1965; Beaumont et al., 1967, 1970; Koga et al., 1974) presented evidence in isolated cases of multiple myelomatosis that this material contained lipids. In all except one of these cases the patients were also hyperlipidaemic (Koga et al., 1974).

Out of our 30 patients with multiple myelomatosis, all normolipidaemic, 24 had abnormal lipid stained bands, which were of three different types of mobility. We confirmed the lipid nature of the sudan-stained material by thin layer chromatography. We found that it was in fact a lipoprotein which was isolated by ultracentrifugation in the bottom fraction with the HDL. In each case the paralipoprotein had a mobility identical with that of the paraprotein. These findings as well as the observation that in one case the disappearance of the abnormal lipoprotein coincided with the disappearance of the paraprotein after treatment favour the view that the lipid carrier was the abnormal protein. This hypothesis, however, fails to explain the absence of p-Lp band in six cases of multiple myelomatosis. We made no attempt to elucidate the nature of the abnormal lipoprotein bands.

Various authors (Beaumont et al., 1967, 1970; Lewis and Page, 1965) presented evidence of a firm complex between the paraprotein and the normal lipoprotein fractions. Nevertheless, in our cases the different migration on the agarose gel of the p-Lp bands in correlation with the normal lipoprotein as well as their high density (> 1.063 g/ml) seem not to support this hypothesis. On the other hand, our findings are compatible with Koga's view that the lipid-containing paraprotein is probably not a result of paraprotein/lipoprotein interaction.

We are continuing our research into the nature of the p-Lp fractions.

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