STUDIES BY QUANTITATIVE IMMUNOELECTROPHORESIS ON IRON BINDING PROTEINS IN HAEMOCHROMATOSIS

A. H. AMIN, H. G. CLARKE, T. FREEMAN, I. M. MURRAY LYON, P. M. SMITH AND R. WILLIAMS

Plasma Protein Section, Clinical Research Centre, Northwick Park, and The M.R.C. Group on Metabolism and Haemodynamics of Liver Disease, King’s College Hospital, London

(Received 17 December 1969)

SUMMARY

1. The concentrations of a number of plasma proteins in patients with haemochromatosis were determined by quantitative immunoelectrophoresis, particular attention being paid to the three iron transporting proteins.

2. Transferrin and haptoglobin were within normal limits; haemopexin was significantly greater than normal ($P<0.001$).

3. Other changes observed were a significant ($P<0.001$) increase of $\alpha_1$ antitrypsin, of $\alpha_2$ macroglobulin and $\beta_{1A-C}$; these changes are discussed.

At least three serum proteins are involved in the ordinary physiological transport of iron. These are, first, transferrin, which carries free iron. In normal circumstances transferrin is only partly saturated, whereas in haemochromatosis it may be $90-100\%$ saturated. Second, haptoglobin; this binds haemoglobin, and although it is known that the mass of haptoglobin catabolized per day is not enough to play a large role in the daily haemoglobin breakdown, it is conceivable that an upset in this mechanism could, over the years, account for an increase in the deposition of iron. And finally, haemopexin transports iron in the form of haem. Very little is known about the metabolism of this protein. It is thought (Müller Eberhard et al., 1969) that haemopexin is a ‘suicidal’ protein like haptoglobin, in other words, after haem has combined with haemopexin the two are catabolized together. There is some evidence to support this hypothesis, in that a low haemopexin is sometimes found in haemolytic conditions; however, the concentration of haemopexin seldom drops as low as is found for haptoglobin.

For these reasons it seemed of importance to study the concentrations of these proteins in primary idiopathic haemochromatosis, a condition associated with a slow accumulation of iron in the tissues and eventual portal cirrhosis, and to compare the concentrations found with those in the normal population. At the same time the concentration of eight other proteins were studied.

Correspondence: Dr T. Freeman, National Institute for Medical Research, Mill Hill, London, N.W.7.
MATERIALS AND METHODS

Twenty-nine patients with primary idiopathic haemochromatosis were studied. The diagnosis of haemochromatosis had been confirmed in all the patients by liver biopsy which showed portal cirrhosis and Grade IV iron deposition according to the criteria of Scheuer, Williams & Muir (1962). Many of them had been under observation for several years. All but one were being or had been treated by weekly venesection for periods up to 3 years prior to the study.

**Protein estimation**

Eleven proteins (α₁ lipoprotein (α₁Lp), α₁ easily precipitable glycoprotein (α₁PGp), α₁ antitrypsin(α₁AT), α₂ Group Component (α₂GC), α₂ macroglobulin (α₂M), caeruloplasmin(Caer), haptoglobin (Hpt), haemopexin (Hpx), transferrin (Trf), β lipoprotein (βLp) and β₁A-c(C’3) were measured according to the technique of quantitative immunoelectrophoresis as modified from Laurell’s initial description (1965) by Clarke & Freeman (1968). Using this technique the area contained under each individual protein curve is proportional to the concentration of that protein in the serum. It is also inversely proportional to the concentration of antibody to that particular protein; thus to make the technique quantitative it is necessary to use a single pool of antiserum throughout the study, and to use some form of standard. In this study the standard used was that originally described (Clarke & Freeman, 1968) obtained from a pool of whole human serum. The areas under the curves were measured using an electronic planimeter designed by J. Lewin of the National Institute for Medical Research, and manufactured by Chemical Electronics Co. (C.W.S. Hall, Durham Road, Birtley, Co. Durham, England). The values used throughout the study as normal were those obtained in the previous study described by Clarke & Freeman (1968).

RESULTS

A photograph of a separation of normal serum is shown in Fig. 1 and that of a haemochromatotic serum is shown in Fig. 2. Table 1 gives the values for the twenty-nine patients with haemochromatosis and 100 normal subjects aged 16–65, giving means and SD. The significant differences obtained between the normal group and patients are shown.

DISCUSSION

The present study has shown highly significant differences in protein concentration between the patients with haemochromatosis and the normal subjects. It should be noted that transferrin, the only protein transporting free iron, is within the normal range. In the light of the concentrations obtained for other proteins, perhaps this is the most remarkable feature of this study. There are no significant correlations between the protein concentrations measured and any of the clinical data, which included duration of venesection therapy and extent of iron overload at the time of measurement. The exact relationship between tissue damage and iron overload is difficult to determine, and recently Smith et al. (1969) were unable to correlate the clinical findings at the time of presentation with the total body iron store measured by the differential ferroxamine technique.

A close relationship between haemopexin and the α₂ Group Component was found (r=0.86,
Fig. 1. A quantitative immunoelectrophoretic analysis of normal serum, showing \( \alpha_1 \)-lipoprotein (\( \alpha_1 \text{Lp} \)), \( \alpha_1 \) easily precipitable glycoprotein (\( \alpha_1 \text{P Gp} \)), \( \alpha_1 \) antitrypsin (\( \alpha_1 \text{AT} \)), \( \alpha_2 \) Group component (\( \alpha_2 \text{GC} \)), \( \alpha_2 \) macroglobulin (\( \alpha_2 \text{M} \)), caeruloplasmin (\( \text{Caer} \)), haptoglobin (\( \text{Hpt} \)), haemopexin (\( \text{Hpx} \)).

(Facing p. 614)

Fig. 2. A quantitative immunoelectrophoretic analysis of serum from a patient with haemochromatosis showing the same proteins as indicated in Table 1.
Iron binding proteins in haemochromatosis

$n=27, P<0.001$). The reason for this is not clear. The function of the $\alpha_2$ Group component is unknown, but similar correlations between these two proteins have been noted in other conditions (Clarke, Freeman & Pryse-Phillips, 1970a). The interpretation of this finding must wait until more is known about the metabolism and function of Group component, and indeed of haemopexin.

Whilst it is appreciated that the measurement of concentration is not a valid way of assessing metabolism of a protein, it would appear from this study that two of the three known iron transporting molecules, transferrin and haptoglobin, are within normal limits, and that haemopexin is significantly above normal. From measurements of concentration alone it cannot be said whether the increased concentration of haemopexin is due to an increase of synthesis or to a decrease in catabolism. Nor can it be said whether the increase is related to the aetiology of haemochromatosis or merely the resultant of a high body iron load at some time.

Significantly high values for haemopexin have been found in other conditions: in active tuberculosis ($P<0.001$) but not in sarcoidosis (Clarke, et al., 1970a); in schizophrenia ($P<0.001$) but not in epilepsy (Clarke, Freeman & Pryse-Phillips, 1970b); and to a lesser extent in rheumatoid arthritis ($P<0.05$) (Clarke, Freeman & Pryse-Phillips, to be published). It could be argued that all four conditions (tuberculosis, schizophrenia, rheumatoid arthritis and haemochromatosis) show protein changes consistent with the ‘acute phase reaction’. This ill-defined term is commonly used to describe that group of proteins which increase in concentration after tissue damage. The concept is supported by an increase in $\alpha_1$ antitrypsin in all four conditions tuberculosis, haemochromatosis and rheumatoid arthritis ($P<0.001$), and schizophrenia ($P<0.05$); however, the changes in concentration in other proteins are not similar, for example caeruloplasmin is increased in tuberculosis ($P<0.001$) and in haemochromatosis ($P<0.01$) but not in rheumatoid arthritis or schizophrenia, and $\alpha_1$ easily precipitable glycoprotein is

<p>| Table 1. Protein concentrations (Mean Values and SD, expressed as per cent reference serum) in twenty-nine patients with haemochromatosis and 100 normal subjects |
|---------------------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Protein</th>
<th>Haemochromatosis</th>
<th>Normals</th>
<th>Statistical significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>$\alpha_1$Lp</td>
<td>139 (46)</td>
<td>127 (20)</td>
<td></td>
</tr>
<tr>
<td>$\alpha_1$PGp</td>
<td>142 (27)</td>
<td>115 (19)</td>
<td></td>
</tr>
<tr>
<td>$\alpha_1$AT</td>
<td>152 (39)</td>
<td>114 (18)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$\alpha_2$GC</td>
<td>138 (41)</td>
<td>117 (15)</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>$\alpha_2$M</td>
<td>215 (68)</td>
<td>122 (25)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Caer</td>
<td>173 (59)</td>
<td>137 (25)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Hpt</td>
<td>113 (71)</td>
<td>103 (44)</td>
<td></td>
</tr>
<tr>
<td>Hpx</td>
<td>156 (49)</td>
<td>107 (13)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Trf</td>
<td>119 (36)</td>
<td>110 (15)</td>
<td></td>
</tr>
<tr>
<td>$\beta$Lp</td>
<td>146 (85)</td>
<td>177 (43)</td>
<td></td>
</tr>
<tr>
<td>$\beta_1\alpha-c$</td>
<td>155 (69)</td>
<td>107 (24)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>n</td>
<td>29</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
normal in haemochromatosis and rheumatoid arthritis but raised in active tuberculosis ($P=0.001$) and to a lesser extent in schizophrenia ($P<0.05$). Finally haptoglobin, which is often quoted as an example of an 'acute phase protein', is normal in haemochromatosis, but increased in tuberculosis (mean 378, $n=16$, $P<0.001$) and rheumatoid arthritis (mean=227, $n=18$, $P<0.001$). It is however conceivable (though we think unlikely) that the normal concentration found for this protein in haemochromatosis is the resultant of increased synthesis ('acute phase') and increased catabolism due to combination with haemoglobin. This point can only be decided by metabolic studies using trace labelled haptoglobin.

The large increase in $\alpha_2$ macroglobulin seen in haemochromatosis is of some interest. Although a controlled study of other diseases with primary liver damage is not yet complete, it seems probable that similar high concentrations will be found. Again it is not clear whether this is due to increased synthesis or to decreased catabolism, though high protein concentrations are usually due to increase in synthesis. High concentrations of $\alpha_2$ macroglobulin are also found in the newborn (Abrams & Freeman, 1969), in children (Abrams, 1970) and in young adults (Clarke & Freeman, 1968). The physiological reason for this is not known. Finally, the high significant increase ($P<0.001$) in concentration of $\beta_{1A-C}$ (the third component of complement: C'3) is unexplained. It would seem likely that this too is consequent to other pathological processes, and is not necessarily related to its complement activity.

ACKNOWLEDGMENTS

One of us (A.H.A.) was in receipt of a training grant from the Sudanese Government, and would like to thank his colleagues in the Department of Chemical Pathology, King's College Hospital, for their help and co-operation. We also wish to thank Miss Lesley Bissett for her skilled technical assistance.

REFERENCES