The serum concentration of the third component of complement $\beta_{1C}/\beta_{1A}$ in liver disease

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SUMMARY Serum $C_3 (\beta_{1C}/\beta_{1A})$ has been measured in normal individuals and the range found is in agreement with findings of other authors (85-370 mg/100 ml). In 18 patients with acute hepatitis and massive necrosis serum $C_3$ was consistently reduced to below 50% of normal. In other patients with acute hepatitis the serum $C_3$ concentration was normal. In the majority of the 150 patients with chronic liver disease serum $C_3$ concentration was normal. However, 10 patients (six with active chronic hepatitis, four with cryptogenic cirrhosis) had hypocomplementaemia. The reason for the depression is not clear but could reflect either decreased synthesis or increased consumption, or a combination of the two.

Complement is a collective term applied to a group of serum proteins which participate in antigen-antibody reactions leading to irreversible cell damage and eventually lysis. Apart from its role in defence it is likely that complement is involved in a variety of disease states. The participation of complement can be assessed in several ways. Immunofluorescent studies can reveal the presence of complement deposited in various diseased tissues. In serum the activity of the complete complement system can be measured. This is usually expressed as the concentration producing 50% lysis of red blood cells. The serum concentration of the third component of complement, $C'_3(\beta_{1C}/\beta_{1A})$, closely reflects the total complement activity (Klempner, Gotoff, Alper, Levin, and Rosen, 1965; West, Northway, and Davis, 1965), and this can now be measured very simply using a radial immunodiffusion technique (Shanbrom, Khoo, and Lou, 1967).

Serum complement has been measured in a variety of diseases, and been found to be reduced in those diseases where there is evidence of antigen-antibody reactions, such as systemic lupus erythematosus (Asherson, 1960; Townes, 1967). Serum complement can also be increased above normal in a wide variety of diseases where there is acute inflammation, and in this situation it behaves as an acute phase reactant (Townes, 1967). The total haemolytic activity of complement has only occasionally been measured in liver disease. Townes (1967) reported that it was reduced in six of nine patients with lupoid hepatitis, and Asherson (1960) found it reduced in a number of patients with hepatitis of varying aetiology.

$C'_3$ has been measured in a number of diseases, and the changes parallel total haemolytic activity. Ogg, Cameron, and White (1968) found that the majority of patients with primary glomerular disease and heavy proteinuria had elevated $C'_3$ concentrations, whilst a significant proportion had reduced levels. The only report of $C_3$ levels in chronic liver disease is that of Mueller-Eckhardt, Kretzschmar, and Kuhn (1970), who failed to find any correlation between serum $C_3$ concentration and the titre of antinuclear antibody in patients with chronic hepatitis. No reference was made to the absolute levels of $C'_3$ in the serum and therefore it is not apparent if any of the patients had reduced levels.

In an epidemic of hepatitis with 300 patients, Mirick (1952) found that 55% showed allergic manifestations, such as urticaria, arthralgia, and angio-neurotic oedema. This extended the observations of others (Hawley, McFarlan, Steigman, McMichael, and Dible, 1944). Marcov-Mutznner (1925) suggested that the jaundice of infectious hepatitis was a manifestation of liver hypersensitivity. Goldgraber and Kirnser (1961) support this concept and in a comprehensive review of the literature suggest that the liver changes might result from allergen-antibody interaction. More recently, Almeida and Waterson (1969) have postulated that antigen-antibody complexes might be important in the pathogenesis of Australia antigen-positive liver disease. In chronic liver disease disturbed immunity has been reported, particularly...
in primary biliary cirrhosis, cryptogenic cirrhosis, and active chronic hepatitis. In view of these observations and the paucity of information on serum complement levels in chronic liver disease it was decided to measure serum C₃ concentration in various types of acute and chronic liver disease.

Methods

Serum C₃(β₁C/β₁A) was measured by radial immunodiffusion using commercially prepared plates (Hyland Laboratories). Most of the sera were tested within 48 hours of collection, and during this time were stored at 4°C. The remaining sera were stored at −20°C for up to three months before testing. Sera and reference standards were placed in wells of equal size in agar plates containing specific antibody against β₁C/β₁A. The plates were incubated at room temperature for 16 hours and during this time a precipitin ring is formed. The diameter of the ring is directly proportional to the serum concentration. Estimations were carried out on batches of 21 to 33 specimens (four to six plates). On each occasion plates with the same batch numbers were used and three reference standards were set up with each run—60, 185, and 360 mg/100 ml. A standard curve was drawn by plotting the precipitin ring diameter of the reference standards on an arithmetic scale against their concentration on a logarithmic scale. This always resulted in a straight line and the individual levels of C₃ are obtained by reference to the standard curve.

The measurements are most accurate when the concentration falls between the highest and lowest standard and the majority of specimens fell within this range. The slope of the standard curve varied very little and only tended to shift to the left or right in parallel. Duplicate estimations were routinely performed and were usually between 4 and 7% of each other, and always within 13%.

Patients

Forty-six healthy blood donors were used as normal controls. Two hundred and twenty-one patients with various forms of liver disease were investigated. There were 49 patients with acute viral hepatitis from the United Kingdom, from two epidemics. The first was an epidemic of hepatitis-associated (Australia) antigen (HAA) positive hepatitis in a renal dialysis unit (Knight, Fox, Ballo, Niazi, Sherlock, and Moorhead, 1970) and the second an epidemic of infectious hepatitis in a community (Ajdukiewicz, Doniach, Dudley, Fox, and Sherlock, 1971). Thirty-four of the 49 patients were positive for hepatitis-associated antigen. In addition there were 18 patients with massive necrosis of the liver; in 15 the diagnosis was fulminant viral hepatitis and four were HAA positive. The remaining three with massive necrosis had recently ingested drugs—paracetamol, marsilid, and isoniazid. Three of the 18 patients with massive necrosis survived, one after exchange transfusion and two following perfusion with the isolated porcine liver. Serial C₃ determinations were performed on these patients.

There were nine patients with normal liver function tests who were carriers of hepatitis-associated antigen. The remaining 150 patients had chronic liver disease and the diagnosis was based upon clinical, biochemical, and histological findings. This group consisted of 11 patients with chronic persistent hepatitis, 53 with active chronic hepatitis, 35 with

\[
\begin{align*}
N &= \text{normals, blood donors} \\
AVH &= \text{acute viral hepatitis} \\
MN &= \text{massive necrosis of the liver} \\
C &= \text{carriers of Australia antigen} \\
CPH &= \text{chronic persistent hepatitis} \\
ACH &= \text{active chronic hepatitis} \\
CC &= \text{cryptogenic cirrhosis} \\
PLC &= \text{primary liver cell cancer} \\
ALD &= \text{alcoholic liver disease} \\
PBC &= \text{primary biliary cirrhosis}
\end{align*}
\]

Fig. 1  Serum C₃ concentration in normal subjects and patients with liver disease
cryptogenic cirrhosis, six with primary liver cell cancer, 15 with alcoholic liver disease, and 30 with primary biliary cirrhosis.

Results

The results are seen in Figure 1. The normal values ranged from 86 to 370 mg/100 ml with a mean (logarithmic) of 170 mg/100 ml. Ogg et al (1968) defined hypocomplementaemia as less than 50% of normal, ie, below 85 mg/100 ml. On the results recorded here this would seem to be reasonable and will not include any normal individuals.

All of the 18 patients with massive necrosis of the liver had hypocomplementaemia, ranging from 25 to 77 mg per 100 ml (mean 46 mg/100 ml). There is no overlap with the normal range and the difference is obviously statistically significant (p < 0.005). There is no difference between the patients with a viral aetiology and a drug aetiology. One patient with acute viral hepatitis had hypocomplementaemia, 58 mg/100 ml. This patient had a protracted clinical course with hepatic decompensation leading to death.

Serial estimations were performed on a number of these patients. In those who died C'3 concentration remained low (patient 1, Fig. 2). One patient, a staff nurse from a renal dialysis unit, was admitted in coma, with hypocomplementaemia of 46 mg/100 ml. She received an exchange transfusion of 10 units of blood, with improvement in her level of consciousness. The serum complement rapidly returned to and remained normal (patient 2, Fig. 2). Two patients survived prolonged hepatic coma after treatment with the isolated perfused porcine liver; the serum complement was markedly reduced in both patients and returned to normal with recovery of liver function (one is illustrated, patient 3, Fig. 2). After perfusion, patient 3 slowly recovered consciousness and three weeks later the serum C'3 returned to the normal range, with concomitant improvement in liver function.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Serum C'3</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Antibody</th>
<th>Medical Status</th>
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<tr>
<td>1</td>
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<td>20</td>
<td>CC</td>
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</tr>
<tr>
<td>2</td>
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<td>F</td>
<td>55</td>
<td>CC</td>
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<td>M</td>
<td>58</td>
<td>CC</td>
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<tr>
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<td>M</td>
<td>34</td>
<td>CC</td>
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<td>5</td>
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<td>M</td>
<td>53</td>
<td>ACH</td>
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<td>60</td>
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<td>M</td>
<td>12</td>
<td>ACH</td>
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</table>

Table  Details of patients with chronic liver disease and reduced complement

1CC = cryptogenic cirrhosis; ACH = active chronic hepatitis; HAA = hepatitis-associated antigen; ANF = antinuclear factor; SMA = smooth muscle antibody; MIT = antimitochondrial antibody; PSE = porto-systemic encephalopathy.

The majority of the 150 patients with chronic liver disease had normal levels of serum C'3. There were no patients with low serum C'3 concentration among those with chronic persistent hepatitis, primary biliary cirrhosis, primary liver cell cancer, or alcoholic liver disease. Six of the 53 with active chronic hepatitis, and four of the 35 with cryptogenic cirrhosis had hypocomplementaemia. Repeated estimations on one of the patients with cryptogenic cirrhosis and on two with active chronic hepatitis showed the hypocomplementaemia persisting. There was no correlation between the level of C'3 and other evidence of disturbed immunity, such as the presence of autoantibodies or elevated serum immunoglobulins. The 10 patients with hypocomplementaemia form a heterogeneous group, and the actual C'3 levels and clinical details are shown in tabular form. There was evidence of liver decompensation in three of the four patients with cryptogenic cirrhosis. One of these patients (4) died shortly after determination of serum C'3, and another (patient 2) is now terminal. In both the serum C'3 remained low. The other two (1 and 3) remain quite
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well with low serum \( C'_3 \); in neither of these is there evidence of increased immunological activity. In one there is evidence of poor liver function with chronic portosystemic encephalopathy.

Three of the six patients with active chronic hepatitis were positive for hepatitis associated antigen, and negative for autoantibodies. All three showed features of hepatic decompensation with jaundice, and ascites in one. The remaining three patients showed varying degrees of liver failure. One (8) died soon after serum \( C'_3 \) was measured and had marked liver failure. The other two have persisting hypocomplementaemia with little evidence of liver failure or activity of liver disease.

Some patients in the different groups of chronic liver disease had serum \( C'_3 \) concentrations above the upper limit of normal (Fig. 1).

Discussion

Many antibodies fix complement in the presence of their corresponding antigen. In certain diseases, such as systemic lupus erythematosus, antigen-antibody combination occurs in vivo and the low serum complement activity is thought to reflect this interaction (Asherson, 1961). In chronic liver disease abnormal autoantibodies have been detected (Walker, Doniach, Roit, and Sherlock, 1965) and disturbed immunity has been suggested as an important aetiological factor in three main forms of chronic liver disease—active chronic hepatitis, cryogenic cirrhosis, and primary biliary cirrhosis. The formation of antigen-antibody complexes within the liver and the subsequent involvement of complement has been suggested as important in the direct cause of liver damage; however, immune complexes have only rarely been located within the human liver (Popper, Paronetto, and Schaffner, 1965).

If antigen-antibody complexes are confirmed in the pathogenesis of liver diseases, such as active chronic hepatitis, the serum complement would be expected to be low in these conditions. In fact, such low values were found in only a small proportion of patients with active chronic hepatitis and cryogenic cirrhosis and not at all in primary biliary cirrhosis. There was no good correlation between serum \( C'_3 \) and activity of disease, although the degree of immunological disturbance is very difficult to assess. Mueller-Eckhardt and his colleagues (1970) failed to find any significant correlation between the titre of antinuclear factor and the level of serum \( C'_3 \). This was not altogether unexpected; indeed one might anticipate a negative correlation. If complexes are being formed and utilizing complement, free antibody will not be found.

The very low values for serum \( C'_3 \) found in massive hepatic necrosis might be due to increased consumption or decreased synthesis, although it should be stated that measurement of serum \( C'_3 \) is a relatively insensitive index of complement consumption. Almeida and Waterson (1969) have suggested that the massive necrosis of fulminant hepatitis might be mediated by liver injury due to hepatitis-associated antigen-antibody complexes and the low results for serum \( C'_3 \) would agree with this hypothesis. However, the liver is the main site of \( C'_3 \) synthesis (Alper, Johnson, Birtch, and Moore, 1969) and it seems more likely that the reduced levels in massive necrosis are due to decreased synthesis. The serum \( C'_3 \) level did seem to be related to the extent of hepatocellular dysfunction and returned to normal as liver function improved. The matter might be clarified by measuring alternative complement components not synthesized by the liver (C1q) or by measurement of the anabolic and catabolic rates for \( C'_3 \).

The findings in chronic liver disease are equally difficult to interpret. Although some of the patients with low complement values did have obvious liver cell failure, there are many other patients with normal serum complement and an equal degree of hepatic decompensation.

The interpretation of reduced complement activity in chronic membranoproliferative glomerulonephritis has also been difficult. There are several factors which contribute to the reduced complement activity which includes the presence in the serum of a complement-inactivating factor (Pickering, Gewurz, and Good, 1968), decreased synthesis, and increased catabolism (Alper and Rosen, 1967). None of these factors have been studied in relation to liver disease.

Measurement of \( C'_3 \) is an easy and a relatively accurate technique and we have shown that levels may be reduced in certain types of liver disease. The place of decreased synthesis and increased utilization is, however, not clear and further study is likely to be of great interest.

References


