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Immune complexes in acute and chronic liver disease

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SUMMARY

Anti-complementary (AC) activity and C1q-binding are increased in acute type A and B hepatitis, alcohol-induced hepatitis, HB surface antigen-positive and -negative chronic active hepatitis and primary biliary cirrhosis.

In acute type A hepatitis, a large increase in C1q-binding was demonstrated during the period of elevated transaminases. In type B hepatitis, the initial peak was small, but was followed by a further peak during the period of falling serum HB surface (HBs) antigen titre. In both diseases the C1q-binding was associated with >20S particles. In paracetamol-induced necrosis, C1q-binding remained normal. In type A and B hepatitis and in paracetamol-induced necrosis, C4, C3 and factor B concentrations were depressed in the early phase of the disease. This change may reflect either diminished synthesis or increased catabolism.

In chronic active hepatitis (HBs-positive and -negative) and in alcohol-induced disease there is a significant correlation between C1q-binding and the severity of hepatitis. C1q-binding and AC activity were also increased in primary biliary cirrhosis. Density gradient studies indicate that the C1q-binding activity in these subjects lies in the 8–14S and > 19S particle-containing fractions.

These findings suggest the presence of immune complexes in patients with acute and chronic liver disease. In some cases the complexes may contained hepatitis viral antigens, but in alcohol-induced and autoimmune disease other types of complex formation must exist. The accumulation of large and small complexes in subjects with liver disease may be a reflection of an impaired function of the mononuclear phagocytes in these diseases. The potential of these complexes to activate complement will determine their pathological importance, and in this respect those found in primary biliary cirrhosis may have special significance.

INTRODUCTION

Immune complexes have been described in patients with HB surface (HBs) antigen-positive acute and chronic hepatitis (Prince & Trepo, 1971; Nydegger *et al.*, 1974). Several studies suggest that viral antigens are contained in the complexes in these diseases (Wands, Alpert & Issilbacher, 1975a; Wands *et al.*, 1975b). The finding of complexes of unknown antigenic composition in HBs antigen-negative chronic active hepatitis (Wands *et al.*, 1975a) raises the possibility that such complexes may result from liver damage and may therefore also be present in other forms of liver disease. For this reason we have examined sera from subjects with acute type A and B viral hepatitis, paracetamol-induced necrosis, HBs antigen-positive and -negative chronic active and persistent hepatitis, various types of alcohol-induced liver injury and primary biliary cirrhosis for the presence of immune complexes. We have used the anti-complementary assay (AC) (Johnson, Mowbray & Porter, 1975), which is highly sensitive, but may measure moieties other than immune complexes, and the C1q precipitation technique of Nydegger *et al.* (1974), which is more specific, measuring only macromolecules which bind C1q, a subunit of the first component of complement.

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MATERIALS AND METHODS

Serial sera from patients with acute type A and B hepatitis and single specimens from patients with primary biliary cirrhosis (eighteen), HBs-positive (twenty-five) and -negative (twenty-four) chronic active hepatitis, HBs-positive chronic persistent hepatitis (nine) and alcohol-related liver disease (twenty-four) (steatosis and hepatitis, with or without cirrhosis) were collected and stored at −20°C until testing within the next 6 months. Control sera from normal subjects (twenty-four)were collected and stored under the same conditions.

The AC activity was assayed by the technique of Johnson *et al.* (1975). This involved the incubation of 0·2 ml of heat-inactivated test serum (30 min at 56°C) with 0·2 ml of a solution containing 2·5 haemolytic units (CH_{50}) of guinea-pig complement for 60 min at 4°C. The remaining complement after incubation was measured by back-titration against sheep erythrocytes optimally sensitized with IgG antibody. The amount of complement consumed was then calculated and expressed in CH_{50} units.

The capacity of the sera to bind radiolabelled C1q was measured by the technique of Nydegger *et al.* (1974). C1q, purified by the modified method of Reid, Lowe & Porter (1972) was radiolabelled with iodine using the chloramine-T method (McConahey & Dixon, 1966). Aggregates of C1q were removed by centrifugation at 50,000 *g* for 30 min before the material was used in the assay. 50 μl of ^{125}I-labelled C1q (1 mg/100 ml; sp. act. 250 μCi/mg of protein) were incubated with an equal volume of the patient's serum for 60 min at room temperature and then 60 min at 4°C. The 'fixed' C1q was then precipitated with a solution of polyethylene glycol (in phosphate-buffered saline at pH 7·4) at a final concentration of 2·5%. The radioactivity bound in the centrifuged precipitate was measured and expressed as a percentage of the total amount of C1q added. Sera from some subjects were run as standards so that comparisons between batches could be made.

Some sera from each disease group were subjected to centrifugation on linear sucrose gradients (10–40% w/w) to determine the sedimentation coefficient of the C1q-binding and AC activities.

In all disease groups the relationship of disease activity to the serological findings was determined. All cases were diagnosed by a combination of clinical, biochemical, serological and histological criteria. Immunoglobulin and complement concentrations were measured by radial immunodiffusion using Hyland plates.

RESULTS

Serial studies were carried out in patients with acute type A or B hepatitis or paracetamol necrosis. In three subjects with type B hepatitis, increased C1q-binding was demonstrated during the hepatitic phase of the illness. In two of these subjects, during the phase of clearance of surface antigen from the peripheral blood and after the serum transaminase level had returned to normal, a second peak of

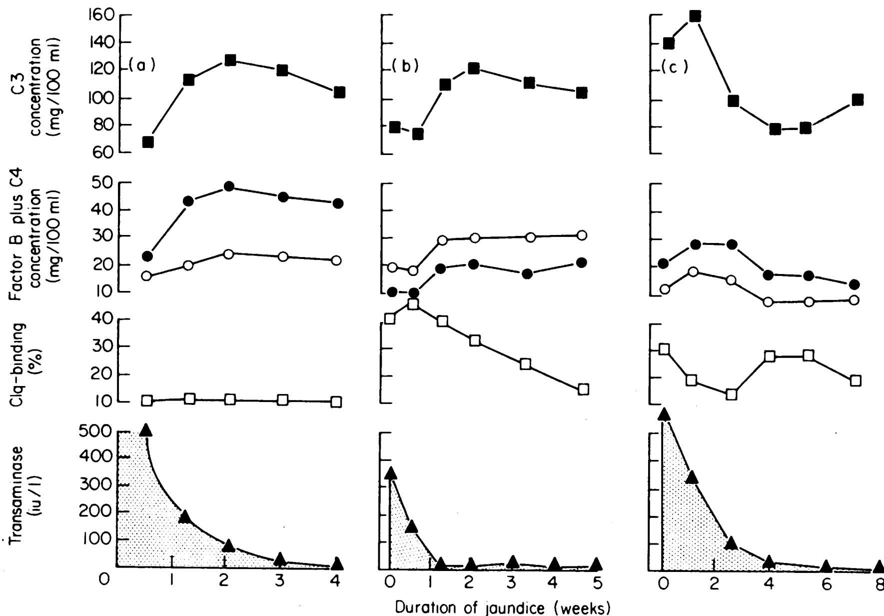

FIG. C1q-binding and complement concentrations in (a) acute paracetamol necrosis, (b) acute type A hepatitis and (c) acute type B hepatitis. (■) C3; (●) C4; (○) factor B; (□) C1q; (▲) transaminase.

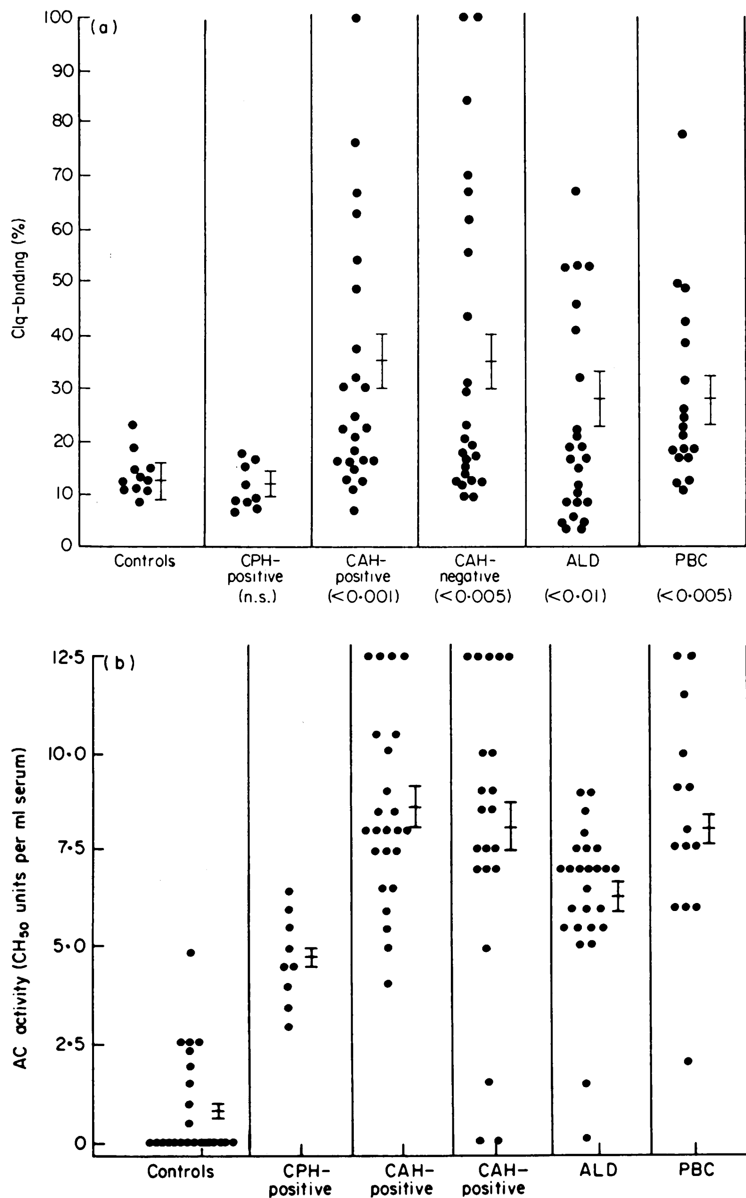

FIG. 2(a) Clq binding in chronic liver disease. *P* values in parentheses. (b) Anti-complementary activity in chronic liver disease. Means ± s.e.m. are shown by $\frac{+}{}$.

Clq-binding occurred (Fig. 1). The third patient was lost to follow-up before this stage. When sera from the patients with increased Clq-binding were examined by electron microscopy, clumps of HB surface antigen were not seen.

In type A hepatitis, the rise in Clq-binding during the hepatitic illness was several times greater than in the acute type B subjects and the second peak did not occur (Fig. 1). In paracetamol-induced necrosis, no change in Clq-binding occurred during the period of study, although the degree of hepatic necrosis indicated by the serum transaminase levels was comparable with that found in the patients with type A and type B hepatitis (Fig. 1). The AC activity was not studied in these patients. Serum C4, C3 and factor B concentrations were low during the early phase of type A and B hepatitis and paracetamol necrosis. This was followed by a secondary over-swing to greater than normal concentrations.

TABLE 1. Relationship of AC activity to C1q-binding

Disease group	Correlation	Number	*P*
CAH (HBs-negative)	0·48	21	< 0·05
CAH (HBs-positive)	0·48	22	< 0·05
CPH (HBs-positive)	0·46	5	n.s.
ALD	0·25	26	n.s.
PBC	0·09	14	n.s.

n.s. = Not significant.

TABLE 2. Relationship of AC activity and C1q-binding to aspartate transaminase

Disease group		AC	C1q
CAH (HBs-negative)			
(n = 13)	r	0·46	0·77
	P	n.s.	< 0·01
CAH (HBs-positive)			
(n = 10)	r	0·07	0·90
	P	n.s.	< 0·01

r = Correlation coefficient; n.s. = not significant.

TABLE 3. Relationship of AC activity and C1q-binding to serum immunoglobulins

		IgM		IgG		IgA	
Disease group		AC	C1q	AC	C1q	AC	C1q
CAH (HBs-negative)	r	0·26	0·44	0·30	0·37	0·12	0·36
(n = 16)	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CAH (HBs-positive)	r	0·09	0·54	0·39	0·54	0·14	0·47
(n = 13)	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ALD	r	0·31	0·29	0·43	0·56	0·63	0·61
(n = 19)	P	n.s.	n.s.	n.s.	< 0·05	< 0·01	< 0·01
PBC	r	0·05	0·69	0·35	0·38	0·03	0·62
(n = 11)	P	n.s.	< 0·01	n.s.	n.s.	n.s.	< 0·05

r = Correlation coefficient; n.s. = not significant.

C1q-binding (Fig. 2a) and AC activity (Fig. 2b) were significantly increased in HBs antigen-positive and -negative chronic active hepatitis (CAH), alcohol-induced liver disease (ALD) and primary biliary cirrhosis (PBC). There was a significant correlation between these two variables in patients with chronic active hepatitis, but not in the other chronic disease groups (Table 1). The range of observations in any one disease group is large. In chronic active hepatitis there is a significant correlation of C1q-binding, but not AC activity, with aspartate transminase (Table 2), but neither variable correlates with serum immunoglobulins (Table 3).

In the alcohol-induced disease group, patients with steatosis or cirrhosis without hepatitis exhibited normal C1q-binding, but significantly increased anti-complementary activity, while those with hepatitis, with or without cirrhosis, showed highly significant increases in both test systems (Fig. 3a and b). There was a significant correlation of data from both systems with serum IgG and IgA concentrations (Table

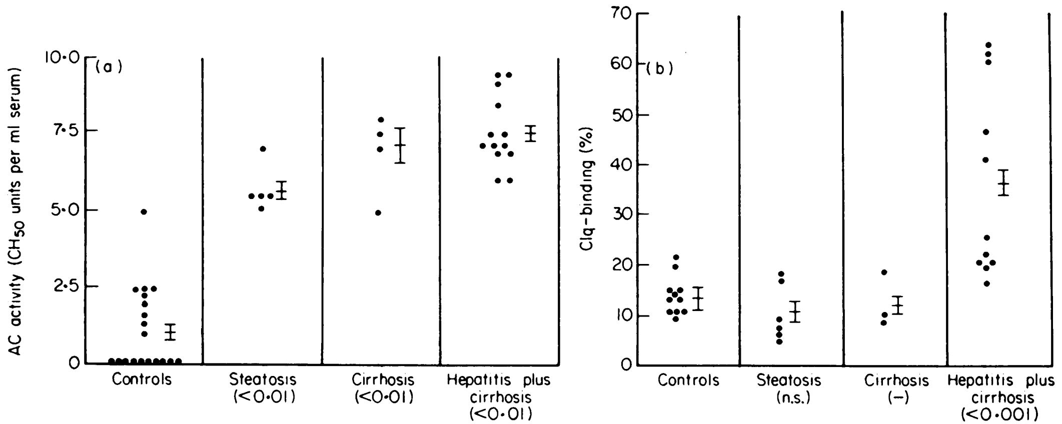

FIG. 3. (a) Anti-complementary activity and (b) Clq-binding in alcohol-induced liver disease. *P* values in parentheses. Mean ± s.e.m. are shown by ⫫.

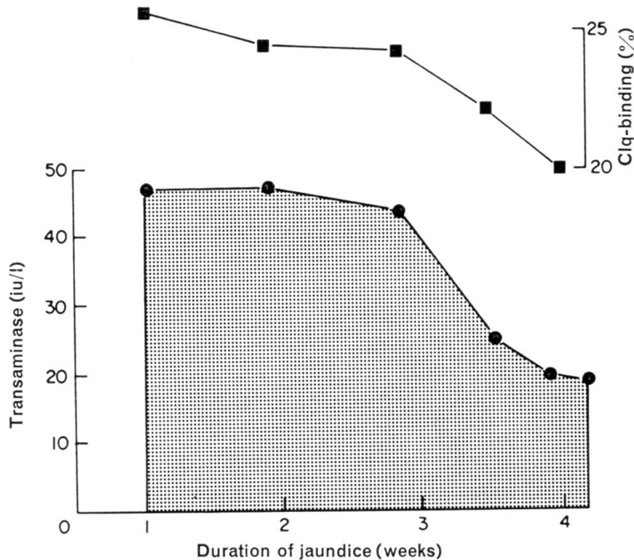

FIG. 4. Serial studies of Clq-binding in alcohol-induced hepatitis. (●) Transaminase; (■) Clq-binding.

3). The association of Clq-binding with alcoholic hepatitis is further substantiated by serial observations in one subject during the recovery phase of this illness. The assay system reflects greatest activity during the early stage of the biopsy-proven hepatitis, with falling values during recovery (Fig. 4).

In patients with primary biliary cirrhosis, increased Clq-binding and AC activity were seen as frequently in stage 1 and 2 as in stage 3 and 4 of the disease (Scheuer, 1972). In this disease group there is a highly significance correlation of Clq-binding, but not AC activity, with the serum concentration of IgM and IgA (Table 3).

Data from sucrose gradients demonstrate that the Clq-binding activity of these sera is associated with large (19–22S) and small (8–14S) molecules (Fig. 5a to d). The larger complexes were more prominent in acute type A and B hepatitis and primary biliary cirrhosis; small complexes were found in all types of disease. Normal sera run on similar gradients did not contain these peaks of activity. The AC assay on these fractions gave increased values not only in subjects with chronic liver disease but also in normal subjects: the interpretation of these data needs further investigation.

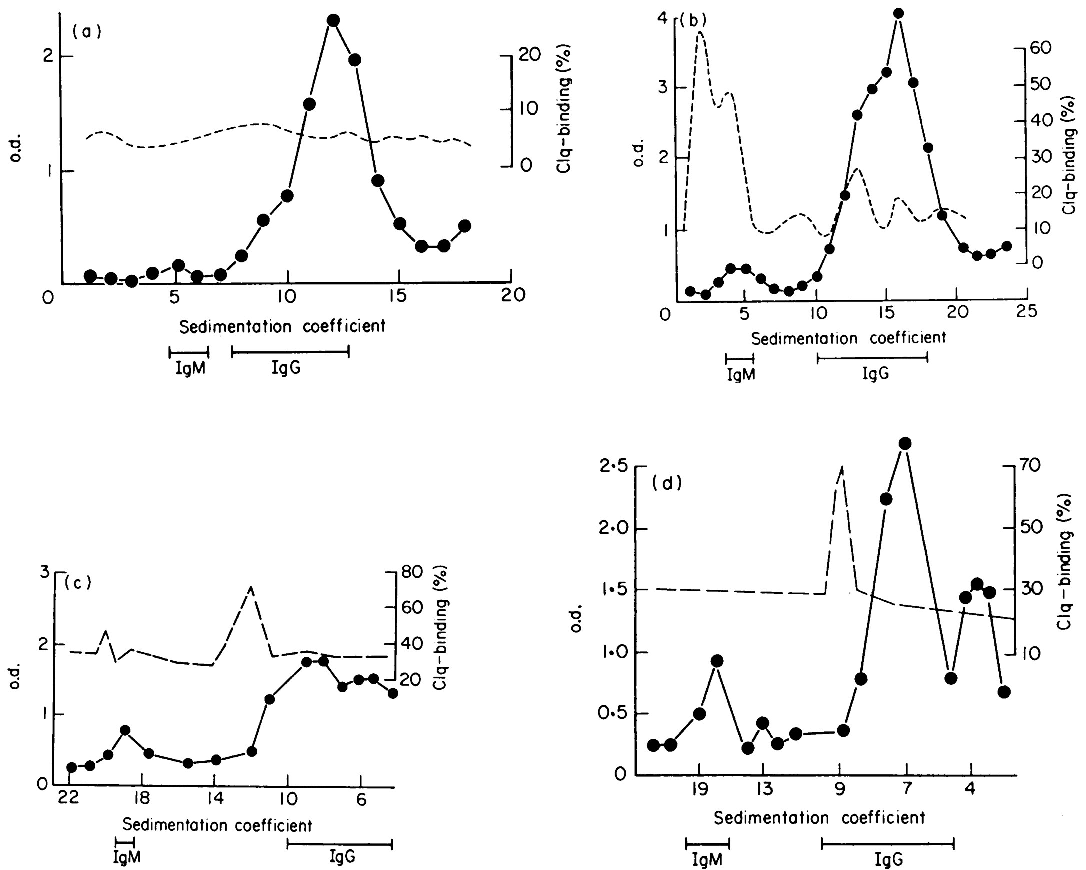

FIG. 5. Determination of sedimentation coefficient of the Clq-binding activity in serum of (a) normal subject, (b) acute type A hepatitis, (c) chronic active hepatitis (HBs Ag-negative), and (d) primary biliary cirrhosis patients. The sera were run on sucrose gradients for 42 hr at 90,000 *g*. Protein content was determined by spectrophotometry; immunoglobulins by immunodiffusion; and the S values were calculated by the method of Funding & Steensgaard (1971). (●) o.d.; (– – –) Clq-binding.

DISCUSSION

Circulating immune complexes may be detected by Clq-binding and AC activity (Johnson *et al.*, 1975; Nydegger *et al.*, 1974). We have detected increased Clq-binding and AC activity in patients with acute and chronic liver disease of varying aetiologies.

Neither of these tests are specific for immune complexes, however, and alternative explanations for these results in patients with liver disease exist. IgG and IgM combine with complement in their native form (Augener *et al.*, 1971) and this binding is enhanced when aggregates or complexes are formed. In some patients with chronic liver disease, therefore, particularly those with primary biliary cirrhosis and high serum IgM levels, hyperglobulinaemia may have contributed to the Clq-binding and AC activity. However, the distribution of Clq-binding within the sucrose density gradient did not correspond to the distribution of IgG or IgM, suggesting that hyperglobulinaemia was not the sole explanation. Circulating endotoxins have been reported in patients with chronic liver disease (Nolan *et al.*, 1974), and although anti-complementary, they do not bind Clq within the conditions of the test (Zubler *et al.*, 1976). The presence of endotoxin might, however, explain some cases in which the results of the two techniques were discrepant. Finally, although variations in the concentration of physiological inhibitors of complement (e.g. $C\bar{1}$ inhibitor or C3b inhibitor) occur in liver disease (unpublished personal

observations), these inhibitors will have been destroyed during the initial heat inactivation of the sera (Gigli, Ruddy & Austin, 1968; Alper, Rosen & Lachmann, 1972).

If the C1q-binding and AC activity in liver disease reflect circulating immune complexes, a number of explanations are possible. Firstly, the hepatic reticuloendothelial system plays a major role in clearing complexes from the circulation (Mannik *et al.*, 1971; Thomas & Vazzedah, 1974) and complexes are not cleared as rapidly as normal in the presence of cirrhosis in experimental animals (Thomas, McSween & White, 1973). Thus immune complexes of whatever origin—post-prandial for example—are likely to persist longer in the circulation in liver disease. The association of increased C1q-binding with active inflammation in type A and type B viral hepatitis, alcoholic hepatitis and chronic active hepatitis might indicate that the reticuloendothelial system is disturbed (or saturated) during episodes of acute hepatic necrosis, but the normal C1q-binding during paracetamol-induced necrosis of comparable severity suggests that this is not always so.

Other authors have indicated that viral antigens are present in circulating complexes during the prodromal phase of acute type B hepatitis (Wands *et al.*, 1975). Although, on electron microscope examination of whole sera from patients with acute type B hepatitis and increased C1q-binding, we did not detect clumped hepatitis B antigen, it seems probable that at least some of the relatively large complexes detected in type A and type B hepatitis contain viral antigens. This is suggested by the late peak of complexes in type B hepatitis, at the time that hepatitis B surface antigen and the associated e antigen are being cleared from the peripheral blood and free antibodies to these antigens have become detectable (Eletheriou *et al.*, 1975). It could also explain why viral hepatitis was associated with circulating complexes whilst drug-induced hepatitis of comparable severity was not.

The importance of complexes in both type A and B acute hepatitis depends on whether they activate complement *in vivo*. The low C4, C3 and factor B concentrations found in this and other studies (Kosmidis & Leader-Williams 1972; Thompson *et al.*, 1973) is compatible with the involvement of complement-mediated tissue damage, but diminished hepatic synthesis of these complement components may also contribute: this is supported in paracetamol necrosis by the low concentrations in the absence of C1q-binding.

In patients with chronic liver disease, who are known to have circulating antibodies that react with hepatic constituents, liver cell antigens may be present in the complexes.

It is possible that circulating immune complexes which may reflect impaired hepatic phagocytic function or the immune clearance of viral antigens or be secondary to autosensitization, play no role in the pathogenesis of liver disease. The almost ubiquitous presence of a variety of complexes in liver diseases of a variety of types might suggest that this was so. There seems little doubt, however, that circulating complexes, in fixing complement, mediate various extrahepatic manifestations of acute and chronic liver disease; furthermore, it may be significant that many patients with primary biliary cirrhosis, in whom we found large complexes to be common, hypercatabolize complement (Potter, Elias & Jones, 1976; Thomas, De Villiers & Sherlocks, 1977) and that large complement-fixing immune complexes cause granuloma formation (Spector & Heeson, 1969). These patients have deposits of complement and immunoglobulin around their bile ducts (Paronetto, Schaffner & Popper, 1967) and further work on the significance of these findings in the primary pathogenesis of this disease is merited.

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