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HBsAg immune complexes in the course of infection with hepatitis B virus

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SUMMARY

Serial serum samples from 113 patients with different forms of HBV-related liver disease and HBsAg carriership were tested for the presence of HBsAg, anti-HBc, anti-HBs, and HBsAg-anti-HBs immune complexes (IC). Eight patients with acute type B hepatitis had the irmultiple serum samples tested in an average period of time from 68 days before the appearance of clinical symptoms up to 277 days after the onset of clinical symptoms. In the remaining cases serum samples were obtained during the period after the appearance of clinical symptoms.

The highest frequency of immune complexes of HBsAg was observed in acute hepatitis (twenty-eight out of thirty examined cases—93·3%). The patients showing high level of anti-HBs response eliminated HBsAg from the circulation earlier than the patients showing low level of anti-HBs response. In chronic aggressive hepatitis the frequency of HBsAg complexes was higher (ten out of twenty-five cases—40%) than in chronic persistent hepatitis (two out of nine cases—22%); HBsAg complexes were found in four out of twenty-two symptomless carriers of HBsAg (18%).

The obtained results are in agreement with the hypothesis that an optimal humoral immune response at the acute stage of hepatitis type B results in rapid elimination of HBV antigens. Conversely, an inadequate response at this stage favours replication of the virus in hepatocytes, prolongation of HBs antigenaemia, and the appearance of chronic forms of hepatitis.

INTRODUCTION

According to the current knowledge, these are major antigenic components of hepatitis B virus (HBV): core antigen (HBcAg), surface antigen (HBsAg), and later discovered *e* antigen (HBeAg). Homologous antibodies anti-two main components, anti-HBs and anti-HBc, were observed in the persons infected with HBV or in convalescents from this infection (Barker *et al.*, 1973; Brzosko *et al.*, 1973; Hoofnagle, Gerety & Barker, 1973; Lander, Alter & Purcell, 1971).

Early observations of the appearance, intermittent presence, and disappearance of HBsAg from blood were interpreted as suggestive for the occurrence of HBsAg in complexes with the homologous antibodies. Immune complexes (IC) of HBsAg were detected in the circulation of patients with acute hepatitis B and various forms of chronic hepatitis by several methods: complement fixation (Purcell *et al.*, 1969; Schmidt & Lennette, 1970; Schulman & Barker, 1969); electron microscopy (Almeida & Waterson, 1969; Stannard *et al.*, 1973); C1q radioimmunoassay (Nydegger *et al.*, 1974); analysis of cryoprecipitates (McIntosh, Koss & Gocke, 1976; Wands *et al.*, 1975); platelet aggregation (Daugharty & Gogel, 1976); and the inhibitory effect on the agglutination of IgG-coated particles by rheumatoid factor or C1q (Lurhuma *et al.*, 1976).

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There have been few observations of the dynamics of the humoral response to the antigens of HBV (Barker *et al.*, 1976; Hoofnagle *et al.*, 1973). This response, if inadequate both quantitatively (antibody level) and qualitatively (antibody affinity) could be a favouring factor in the persistence of viral infection (Eddleston & Williams, 1974; Mims, 1974; Oldstone, 1975). The purpose of this work was to investigate the dynamics of formation and elimination of HBsAg IC in the course of acute hepatitis type B and the incidence of these complexes in different forms of chronic hepatitis.

MATERIALS AND METHODS

The following groups of patients were investigated: (1) thirty patients with acute hepatitis type B, (2) three patients with fulminant and subacute hepatitis, (3) nine patients with chronic persistent hepatitis, (4) twenty-five patients with chronic aggressive hepatitis, (5) ten patients with post-necrotic liver cirrhosis, (6) twenty-two symptomless carriers of HBsAg, and (7) fourteen symptomless carriers of anti-HBs antibodies. The group of acute hepatitis patients included eight members of staff of the Renal Dialysis Unit under observation in the King's County Hospital, Brooklyn, New York, USA. The remaining twenty-two patients with acute hepatitis and the other groups of patients were hospitalized in the Infectious Diseases Institute, Warsaw Medical School, Warsaw, and in the Clinic of Gastroenterology, Postgraduate Medical School, Warsaw.

In all these patients infection with HBV was recognized on the basis of the presence of at least two out of three serological markers: HBsAg, anti-HBs and anti-HBc. The control group consisted of serum samples from fifty blood donors provided by the Blood Bank of the Institute of Haematology, Warsaw. These serum samples were repeatedly negative for HBsAg by counterelectrophoresis and showed normal values for SGOT, SGPT (4–40 iu), and bilirubin (up to 1 mg %).

Sera from twenty-two patients with acute hepatitis were taken at the following periods of time from the appearance of clinical signs of the disease. First sample: 2–18 days (mean 8 days); second sample: 22–60 days (mean 38 days); third sample: 80–200 days (mean 114 days). The most distinct signs of liver damage were observed during initial period of the disease (8 days); mean SGPT value: 804 iu; mean bilirubin level: 8·78 mg %.

During the intermediate period of the disease (+38 days) a decrease of the values of these indices was observed; mean SGPT values: 147 iu; mean bilirubin level: 1·78 mg %. During the final convalescent stage of the disease (+114 days) these values were almost normal; SGPT: 49 iu; bilirubin level: 0·73 mg %.

Samples of sera from eight patients with acute type B hepatitis from among the staff of the Renal Dialysis Unit were available before the appearance of clinical symptoms and then at 6–35-day intervals during the acute phase, and at 150-day intervals during the recovery period.

The diagnosis of the different forms of chronic hepatitis was based on the histologic evaluation of at least two liver biopsies. Liver function tests and the presence of indices of HBV infection were evaluated in the serum samples taken from these patients at 3–4 months intervals during 2 yr.

The persons in whom HBsAg or homologous antibodies was found to persist for at least 6 months were considered symptomless carriers of HBsAg and anti-HBs, respectively, as they did not show either clinical signs or histological or biochemical evidence of liver damage.

The following determinations were made in the collected serum samples:

1. The presence of HBsAg by counterelectophoresis (CEP; Prince & Burke, 1970), and in most cases also quantitative measurement of HBsAg by radioimmunoassay with polyethylene glycol 6000 (RIA-PEG; Lambert *et al.*, 1974).

2. The presence and level of anti-HBs antibodies by radioelectrocomplexing (REC; Madalinski, Gajewski & Walicka, 1974; Simons, 1973).

3. The presence and titre of anti-HBc antibodies by indirect immunofluorescence (IFL; Madalinski *et al.*, 1976).

4. The level of C3 component of complement by radial immunodiffusion method (Mancini, Carbonara & Heremans, 1965).

5. The presence of immune complexes of HBsAg by three different methods: (a) Serum anticomplementary activity (AC)–diagnosed by fixation of at least 3·0 units of heterologous complement by serum diluted 1 : 20 with 0·147 M veronal buffer, pH 7·4, reversible by the addition of HBsAg containing and/or anti-HBs-containing serum (Kabat & Mayer, 1961; Shulman *et al.*, 1964). (b) Radioimmunoassay method measuring the amount of HBsAg bound with the antibodies of IgG class (RIA-IgG). This method is a modification of a radioimmunoassay for the detection of HBsAg using polyethylene glycol 6000 (Lambert *et al.*, 1974). Briefly, IgG was isolated from guinea-pig anti-HBs antisera purchased from Abbott Co. (Chicago, Illinois), as a preparation labelled with ^{125}iodine. Fab fragments from isolated IgG of these antisera were obtained by digestion with mercuripapain (at a protein : enzyme-relative concentration 100 : 1) and purified by chromatography in acetate buffer, pH 5·5 on DEAE-cellulose column.

Serum under test was diluted 1 : 1 with 0·1 M borate buffer, pH 8·6, and the volume of 100 µl was taken and mixed with 100 µl of ^{125}I Fab anti-HBs reagent, incubated 1 hr at 37°C and 3 hr at room temperature then 0·4 ml of rabbit anti-IgG was added. The mixture was incubated 1 hr at 37°C and 18 hr at room temperature then centrifuged at 1000 *g* during 20 min. The precipitate was washed once with 2 ml of 0·1 M borate buffer and its radioactivity was measured in a Packard gamma counter.

The amount of rabbit anti-IgG antiserum needed to precipitate 95% of IgG from normal human serum was determined by precipitation of ^{125}I-labelled IgG.

The precentage of ^{125}I Fab precipitated under these conditions (complex-bound HBsAg) was compared with the percentage of ^{125}I Fab precipitated in RIA-PEG method (total HBsAg).

(c) REC—showing the coexistence of anti-HBs with HBsAg in the same serum sample, and purification of HBsAg and its labelling with ^{125}I for the REC method.

HBsAg (adw) was purified by passing the HBsAg-positive serum sample through the goat globulins anti-HBs- (titre 1 : 64 in immunodiffusion) -Sepharose 4B immunoadsorption column (Cuatrecasas, Wilchek & Anfinsen, 1973) and eluting the antigen with 0·15 M glycine-HCl buffer, pH 1·8. The HBsAg-positive fractions were subjected to further purificaton by rate zonal ultracentrifugations in caesium chloride gradient (Beckman 2L 65B centrifuge, 88,000 *g*, during 6 hr; (Gerin, Holland & Purcell, 1971). HBsAg obtained thus with a protein content 146 μg/ml by spectrophotometry at 280 nm was tested for purity by immunodiffusion and immunoelectrophoresis with anti-HBs and anti-normal human serum proteins (anti-NHS) antisera. The reaction with anti-NHS antisera was negative, but with anti-HBs it was positive. The purified preparation of HBsAg was negatively stained with 3% phosphotungstic acid, pH 4·0 (Almeida & Waterson, 1969) and examined in a JEM 6C electron microscope at 80 kV.

A sample of HBsAg was labelled with 1 mCi of ^{125}I by the method of McConahey & Dixon (1966) with slight modifications consisting in the prolongation (8 min) of chloramine T action, and increasing the dose (200 μg) of sodium persulphate. After dialysis against phosphate-buffered 0·15 M NaCl, pH 7·6 (PBS), the labelled antigen was precipitated in 95% by 20% trichloroacetic acid.

6. Statistical analysis. Patient groups were compared by chi-squared analysis; significance levels were assessed at $P < 0{\cdot}05$.

RESULTS

(1) Patients with acute hepatitis B—twenty-two cases (Warsaw). The highest frequency of HBsAg in serum was found at the first examination (21/22 = 95·5%). One patient had precipitating, CEP-positive anti-HBs in the first sample. At the second examination the frequency of HBsAg incidence was lower (17/22 = 77·3%), and became significantly lower at the third examination (8/22 = 36·4%). In the majority of the latter positive samples, HBsAg was detectable only by RIA-PEG, the most sensitive method used.

The mean level of C3 component of complement in the sera of these patients was 114±63 mg % in the first sample, 156±58 mg % in the second, and 172±43 mg % in the third sample. In the control group mean level of C3 was 145±38 mg %.

Serum anticomplementary activity (12–16 units of complement bound most frequently) was found in 5/22 = 22·76% cases in the first sample, 10/22 = 45·5% in the second, and 8/22 = 36·4% cases in the third sample. However, reversible anticomplementary activity, i.e. activity neutralized by the addition of serum positive for either HBsAg or anti-HBs, was observed in 0, 6, and 4 cases in the first, second and third samples, respectively. Anti-HBc antibodies were observed in all these patients.

The preparation of HBsAg used for the REC method contained particles mostly about 22 nm with occasional tubular forms and Dane particles, as checked by electron microscopy. The level of anti-HBs antibodies was calculated from the results obtained by the REC. The more antibodies contained in the serum under test, the lower value of REC index. The mean value of REC index for serum samples of healthy donors, which did not contain anti-HBs was 5·70 units; the standard error of the mean was 0·90, and confidence limits with 99·9% probability were1·93–9·47. Taking the mean value of 5·70 units as the basis, such sera were considered positive for anti-HBs antibodies, whose REC index was lower than the lower level of confidence zone (1·93). Dilution 1 : 2560 in PBS of goat anti-HBs antiserum gave a value of 0·81 units.

The twenty-two cases of acute hepatitis type B analysed, hospitalized in the Infectious Disease Institute, could be divided into two groups on the basis of arbitrarily chosen levels of anti-HBs antibodies (Table 1). The first group with high level of anti-HBs and high percentage of IC at the first stage of the disease, eliminated most of the HBsAg from the circulation during the observation period. The second with a low level of anti-HBs antibodies, or none at all, at the first stage of the disease and a slowly increasing percentage of IC showed prolonged elimination of HBsAg: five out of seven patients did not eliminate HBsAg during the observation period and three out of these patients developed chronic liver disease.

(2) Patients with acute hepatitis B from among the staff of Renal Dialysis Unit—eight cases (New York). The results obtained in these patients are summarized in Table 2. HBsAg was detected for

TABLE 1. Level of HBsAg anti-HBs, antibodies, and complexes of HBsAg of HBsAg in two groups of patients with acute hepatitis B

	HBsAg PEG units	Anti-HBs	Complexes of HBsAg (percentage of total HBsAg)
Group I ($n = 15$)			
First sample	25·3±11·7	1·04±0·9	23·5
Second sample	17·7±11·7	1·42±0·96	30·1
Third sample	6·7± 6·2	1·91±1·10	2·7
Group II ($n = 7$)			
First sample	34·9±2·38	2·38±0·48	9·0
Second sample	25·6±13·7	3·02±1·19	19·4
Third sample	19·7±14·7	3·51±1·36	34·1

Levels of anti-HBs antibodies arbitrarily chosen from REC index: 0·03–1·25 high level of anti-HBs; 1·251–1·93 low level of anti-HBs; < 1·93 no antibodies.

the first time on or about the sixteenth day before the appearance of clinical symptoms and antigenaemia persisted for 54 days (from 26 to 121 days). Anti-HBs antibodies were detected 29 days before the onset of clinical symptoms and were observed 225 days thereafter. Samples earlier than 29 days before clinical symptoms were negative for anti-HBs. Anti-HBc antibodies were first detected on or about the second day before the occurrence of the symptoms. The highest titre (1 : 3200–1 : 204, 800) was found on the nineteenth day after the appearance of clinical symptoms and these antibodies were still observed on day 277 after the appearance of clinical symptoms at the mean titre of 1 : 500 (1 : 40–1 : 800). Anti-complementary activity reversible by the addition of excess of HBsAg and/or anti-HBs was detected in

TABLE 2. The occurrence of HBsAg, anti-HBs and anti-HBc antibodies in cases of acute hepatitis from among the staff of the dialysis unit

	HBsAg			Anti-HBs		Anti-HBc		
No. of case	Found for the first time	Found for the last time	The number of days with antigenaemia	Found for the first time	Found for the last time	Found for the first time	The highest titre	The final titre
1	−27	0	27	−56	+263	−26	1 : 25,600 (+22)	1 : 40 (+428)
2	−31	+40	71	−98	+287	−20	1 : 6,400 (+15)	1 : 200 (+287)
3	−26	+95	121	+1	+438	−55	1 : 6,400 (+8)	1 : 100 (+538)
4	− 7	+33	40	−11	+ 38	−16	1 : 204,800 (−4)	1 : 800 (+103)
5	− 6	+49	55	−21	+166	+28	1 : 12,800 (+28)	1 : 800 (+188)
6	0	+59	59	+20	+257	+20	1 : 3,200 (+20)	1 : 800 (+301)
7	− 8	+18	26	−27	+148	+22	1 : 3,200 (+27)	1 : 800 (+148)
8	−25	+10	35	−43	+200	+35	1 : 6,400 (+35)	1 : 400 (+221)
Average	−16	+38	54	−29	+225	− 2	+19	1 : 500 (+277)

— Days before the appearance of jaundice; + days after the appearance of jaundice. The samples from the time of appearance of jaundice and 10 days before were not available for anti-HBc.

two cases (No. 5 and No. 6) at the beginning of HBs antigenaemia, in one case (No. 3) twice during the decrease of HBs-antigenaemia and in the remaining cases (Nos. 1, 2, 4, 7, 8) at the end of the observation period.

(3) The occurrence of immune complexes of HBsAg in acute hepatitis and in the remaining groups of patients. The summary of results obtained in acute hepatitis, different forms of chronic hepatitis and in symptomless carriers of HBsAg and carriers of anti-HBs is given in Table 3.

The highest frequency of immunological complexes of HBsAg (detectable by at least two out of three methods used) was observed in the course of acute hepatitis (28/30 cases = 93·3%). In the group of patients with subacute and fulminant hepatitis, complexes of HBsAg were observed in one out of three cases, in the group of patients with chronic aggressive hepatitis in ten out of twenty-five cases (40%), in the group of patients with chronic persistent hepatitis—in two out of nine cases (22%), and in the group of carriers of HBsAg—in four out of twenty-two cases (18%).

TABLE 3. Occurrence of HBsAg, anti-HBs, anti-HBc and HBsAg IC in patients studied and in the control group

Diagnosis	Number tested	HBsAg	Anti-HBs	Anti-HBc	HBsAg IC	
		Radio-immunoassay	Radioelectro-complexing positive	Immuno-fluorescence tested	HBsAg IC	*P*
1. Acute hepatitis	30	29/30	28/30	30/30	28/30	
		96·7%	93·3%	100%	93·3%	significant
2. Chronic aggressive hepatitis	25	18/25	16/25	23/25	10/25	$P < 0·05$
		72%	64%	92%	40%	
3. Fulminant and subacute hepatitis	3	3/3	1/3	2/3	1/3	
					33·3%	
4. Chronic persistent hepatitis	9	8/9	2/9	9/9	2/9	
		89%	22%	100%	22·2%	
5. Symptomless carriers of HBsAg	22	22/22	4/22	22/22	4/22	non-significant
		100%	18%	100%	18·2%	$P < 0·55$
6. Post-necrotic liver cirrhosis	10	5/10	4/10	10/10	0	
		50%	40%	100%		
7. Carriers of anti-HBs	14	0/14	14/14	10/14	0	
			100%	71·4%	0	
8. Blood donors (control group)	50	0/50	6/50	7/50	0	
			12%	14%		

Statistical analysis has shown, that the difference in the frequency of complexes of HBsAg between the group of acute hepatitis and the group of chronic aggressive hepatitis was significant; the difference between the group of chronic aggressive hepatitis and the remaining groups investigated was also significant ($P < 0·05$, Table 3).

The results obtained by quantitative REC method were used for the comparison of the anti-HBs levels during the course of acute hepatitis, chronic forms of hepatitis, and symptomless HBsAg carriership. High level of anti-HBs antibodies (0·03–1·25 units by REC) was found in the group of chronic aggressive hepatitis patients as compared with the group of chronic persistent hepatitis patients and HBsAg carriers, whose sera contained low levels of anti-HBs (1·25–1·93 units by REC).

In fourteen carriers of anti-HBs antibodies and in ten patients with post-necrotic liver cirrhosis complexes of HBsAg were not observed (Table 3). In control group of fifty healthy blood donors anti-HBs antibodies were detected in six persons (12%) and anti-HBc antibodies in seven persons (14%); HBsAg IC were absent.

DISCUSSION

The infection of man with hepatitis B virus closely resembles the persistent viral infections of animals: lymphocytic choriomeningitis, Aleutian disease of mink, lactic dehydrogenase elevating virus, African swine fever, equine infectious anaemia, mouse leukaemia virus, and mink encephalopathy (Hotchin, 1971; Nydegger *et al.*, 1974). In all these infections, persistent release of the excess of viral antigens induces immune response of the host, with the appearance of pathogenic infectious virus–antibody complexes (Notkins *et al.*, 1968; Oldstone & Dixon, 1971). The neutralizing antibodies were detected in the circulation of these animals only after application of sensitive analytical methods. In these animals antibody response of apparent low affinity is insufficient to cause elimination of viral antigens and the virus itself, but it promotes development of phlogogenic immune complexes (Oldstone, 1975). On the other hand, in well-responding animals, humoral response, cellular immunity and interferon take part in the elimination of viral infection.

In the present work the frequency of occurrence of HBsAg, anti-HBs, anti-HBc and immunological complexes of HBsAg was studied in sera of patients with various forms of acute and chronic hepatitis and in carriers of HBsAg or anti-HBs. The infection of these persons with HBV was established by the presence of at least two out of three serological markers: HBsAg, anti-HBs and anti-HBc; with a four-fold increase of the latter two factors during acute hepatitis.

HBsAg was searched for by counterelectrophoresis and PEG radioimmunoassay; anti-HBc—by immunofluorescence. The presence of anti-HBs antibodies was established mainly by the radioelectro-complexing method, which detects the primary binding of antigen by antibody (Simons, 1973). The most important prerequisite for the adequacy of results obtained with REC is the purity of the isotope-labelled antigen. HBsAg was isolated from serum of a symptomless carrier by the two-stage technique, consisting of immunoadsorption column and isopycnic centrifugations in caesium chloride gradient. The obtained preparation of HBsAg was free from normal human serum proteins, as demonstrated by the immunodiffusion and counterelectrophoresis methods.

The highest frequency of HBsAg IC was observed in patients with acute hepatitis (93·3%) and in patients with chronic aggressive hepatitis (40%). Lower frequency of IC was observed in the group of patients with chronic persistent hepatitis (22·2%) and in carriers of HBsAg (18·2%). IC were not found in patients with post-necrotic liver cirrhosis (presumably related to hepatitis B), in anti-HBs antibody carriers, or in the control group.

In a group encompassing 130 patients with type B acute hepatitis studied by Shulman & Barker (1969) serum anticomplementary activity was found in 95% of the cases. However, their sera were kept frozen for a long time, and their criteria of the reversibility of anticomplementary reaction by the addition of HBsAg or anti-HBs are doubtful. The cryoprecipitates isolated by McIntosh *et al.* (1976) from the sera of patients with acute and chronic type B hepatitis contained HBsAg, anti-HBs, or, most frequently, both. This proves that circulating complexes of HBsAg occur in hepatitis patients.

It was suggested that such manifestations of hepatitis as myalgia, rash, arthritis, splenomegaly, nephritis and thrombocytopenia may well arise from circulating soluble HBsAg IC (Kohler, 1973). Elimination of IC from the circulation is accomplished by macrophages, monocytes, neutrophils and fixed phagocytes of the reticuloendothelial system. On the surface of some of these cells—macrophages and monocytes—are found receptors for Fc fragment of IgG and C3 component of complement, which attach immune complexes (Nussenzweig *et al.*, 1971).

The results of our studies indicate that examination of serial serum samples from each patient enables the finding of IC by two radioimmunoassays and complement fixation method in the vast majority of cases of acute hepatitis type B (93·3%). However, only in part of these cases the anticomplementary activity was reversible by adding an excess of HBsAg and/or anti-HBs. The highest frequency of reversible anticomplementary activity (six out of ten anticomplementary samples) was noted at the second examination (+38 days from the onset of acute hepatitis). This may indicate that at this stage of the disease the antigen–antibody ratio approaches equivalence after the first stage of antigen excess. The decrease of the C3 component of complement during acute hepatitis, especially during the first stage of

the disease, was likely connected with its increased binding to immune complexes of HBsAg, not only with decreased synthesis of this protein due to the liver damage (Kosmidis & Leader-Williams, 1972).

The incidence of circulating HBsAg IC in various forms of hepatitis found in this study is lower than that revealed by immunomorphological studies in 101 necropsy cases (Nowosławski *et al.*, 1975). HBsAg IC bound in tissues were identified with similar frequency in patients with acute fatal hepatitis (fifteen out of seventeen cases; 88·8%), but with a higher frequency in fulminant hepatitis (seventeen out of nineteen cases; 89·5%), subacute hepatitis (twenty-four out of twenty-seven cases; 88·2%), in patients with chronic aggressive hepatitis (eight out of thirteen cases; 51·6%), liver cirrhosis (eleven out of twenty cases; 55%), and 'minimal' hepatitis (two out of five cases; 40%).

Trepo *et al.* (1976) tested sixty-four cases of fulminant hepatitis and found circulating complexes of HBsAg and anti-HBs antibodies in 25% and 40·6%, respectively. HBsAg was found in the sera of 59·3% of these patients by radioimmunoassay and its mean persistence time was 5·2 days only (thirteen patients were alive at the end of the study), while HBsAg persistence time in self-limited acute hepatitis was 67 days (54 days in our dialysis unit cases).

The following factors may influence the type of acute attack of hepatitis B and development of chronicity: the dose of antigen (HBV) received; the virulence of the strain; the kind of treatment and the impaired cellular and humoral response of the host (Sherlock, 1976).

In a group of ninety-nine patients with HBsAg-positive hepatitis studied in Denmark, the antigen was cleared within 13 weeks (91 days) in eighty-eight patients, but in eleven patients it persisted. In ten of those with persistent antigenaemia, biopsy showed some form of chronic hepatitis (Nielsen *et al.*, 1971). One of the prognostic markers of the development of chronicity appears to be the persistence of HB*e*Ag (Nielsen, Dietrichson & Juhl, 1974).

In this study it was found that the intensity of the humoral response, especially early formation of anti-HBs which resulted in HBsAg IC, favours elimination of HBV from the organism. The presence of high level of anti-HBs antibodies during the first stage of acute hepatitis is a good prognostic sign suggestive of prompt elimination of the virus from the organism and termination of the infection. On the other hand, our data indicate that the presence of low level of anti-HBs antibodies during the acute stage of hepatitis favours continued replication of the virus in hepatocytes and evolution of the disease into chronicity. The few exceptions to this rule which were noted may be explained by other elimination mechanisms (independent T-cell response, interferon response).

It was also shown that the aggressive form of chronic hepatitis is characterized by a higher frequency of IC and a higher level of anti-HBs than the persistent form of this disease. This is in agreement with the view that these two disease entities differ in the intensity of cellular and humoral response to the antigens of HBV (Dudley, Fox & Sherlock, 1974).

Nydegger *et al.* (1974) and Levinsky, Cameron & Soothill (1977) have shown that levels of IC in patients with systemic lupus erythematosus (SLE) correlated significantly with clinical activity of the disease. The latter authors also suggest that differences in the size of complexes may account for different clinical manifestations of SLE. The patients with renal signs of the disease had predominantly medium-sized IgG complexes ($1·0–1·5 \times 10^6$ mol wt) whereas the patients with the extrarenal manifestations had only very large IgG complexes ($2·5–4·0 \times 10^6$ mol wt). It appears, therefore, that further work would be needed to establish the size and molecular composition of IC in various forms of hepatitis type B and to correlate these data with the disease activity.

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