Haemagglutination in acute hepatitis and other diseases

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SYNOPSIS A haemagglutination test using day-old chick erythrocytes was applied to the sera of 210 patients with hepato-biliary disease and to those of 31 with other diseases.

Thirty-six per cent of 58 patients with infective hepatitis gave positive results and this increased to 60% when sera taken during the first 14 days of symptoms were examined. Three of eight patients with infective mononucleosis gave positive titres. Results were consistently negative in 12 patients with acute obstructive jaundice and a raised titre excludes this condition. Positive results were seen in four of 14 patients with cirrhosis of the alcoholic, in 10 of 43 with non-alcoholic cirrhosis, in one of 11 with acute juvenile cirrhosis, in two of four with haemochromatosis, and in two of 18 with primary biliary cirrhosis. Five of 19 patients with rheumatoid arthritis gave raised titres.

The study of infectious hepatitis has always been handicapped by the absence of an immunological test, not only for the diagnosis of doubtful cases but also for the screening of blood donors. The possibility of a heterophile antibody being produced, analogous to the Paul-Bunnell reaction of infectious mononucleosis, has often been suggested. In cases of hepatitis several studies have been made of heterogenetic antibody which agglutinates erythrocytes of different animal species, including *M. rhesus* erythrocytes and chick erythrocytes (Eaton, Murphy, and Hanford, 1944; Hoyt and Morrison, 1956; Rubin, Kemp, and Bennett, 1957; Morrison, St. Clair, Hoyt, and Stevens, 1958). Following the demonstration of specific yellow fever haemagglutinins in monkey sera treated with acetone and ether (Porterfield, 1954) a similar method was used by Havens (1958) on sera from cases of infective hepatitis to demonstrate the presence of a substance which agglutinated day-old chick erythrocytes in the cold.

McCollum, Bech, Isaacson, and Riordan (1959) confirmed the presence of an agglutinin in 40 to 89% of cases of infectious hepatitis, depending upon the stage of the disease process, the percentage being higher in the early stages. The present investigation was carried out to study the incidence of this haem-agglutinating factor in the sera of patients with infectious hepatitis and hepatic and other diseases.

MATERIALS AND METHODS

SERA Sera were obtained from a total of 252 patients, and in some instances (Table III) several samples were obtained at intervals.

SERUM EXTRACTS Haemagglutination tests were carried out on serum fractions obtained by treating serum with acetone and ether. The serum fractions were twice extracted with chilled acetone and twice with ether by the method described by Havens (1958), and the precipitates so obtained were stored in dry ice until required.

HAEMAGGLUTINATION TESTS These were carried out by the method of Havens (1958), using day-old chick erythrocytes. The serum precipitates were dissolved in 0.15 M NaCl, two-fold dilutions made in borate saline, and 0.5 ml. of dilution mixed with 0.5 ml. of 0.25% washed chick cell suspension. The tests were read after 18 hours at 4°. Six control positive sera were kindly sent to us by Dr. Paul Havens, and either one or two of these samples were used in every batch of tests.

RECORD OF READINGS The results were recorded as follows:—

Negative A smooth-edged button of cells or a smooth-edged ring of cells was recorded as negative.

Positive (a) Clumps of scattered cells were recorded as positive or (b) sediment packed in a ring or button with a crenated edge, sometimes with clumps scattered at the periphery; $\pm$ was a tight button of cells with a crenated edge. A titre of 1:80 or greater was regarded as significant in interpretation of the results. Table I shows

TABLE I

RESULTS OF HAEMAGGLUTINATION TESTS ON
POSITIVE CONTROL SERA

Positive Control Sera	Titres Obtained on Different Occasions at the Royal Free Hospital						Titre Obtained by Havens
A	1:160	1:320	1:320	1:320	1:160	1:320	1:80
B	1:160	1:160	1:160	—	—	—	1:160
C	1:320	1:640	1:320	1:320	1:160	1:640	1:320
D	1:160	1:80	1:160	1:160	—	—	1:160
E	1:160	1:160	1:160	1:160	1:320	>1:80	1:160

the titres obtained with five positive control sera on the various occasions when each was tested, compared with those found by Dr. Havens.

These results show that the endpoint readings obtained in our laboratory were either the same as, or one tube higher, than those of Dr. Havens, except on two occasions (sera D and E). It is of some importance to establish this point because of the relatively low percentage of positive results which was obtained in our laboratory from cases of infectious hepatitis.

RESULTS

The results are presented in Table II.

Fifty-eight cases of infective hepatitis were studied: 21 showed titres of 1:80 or higher. When the results were related to duration of symptoms it was seen that seven of 13 were positive in the first two weeks, but only three of the 13 cases were positive when tested later (Table III). Two of three cases of infective mononucleosis gave positive results in the first two weeks after symptoms but all were negative within 10 weeks. Twelve cases of acute obstructive jaundice

TABLE III

RELATION OF SERIAL RESULTS TO DURATION OF SYMPTOMS
IN INFECTIVE HEPATITIS AND INFECTIVE MONONUCLEOSIS

Condition	No. of Patients	Positive in	
		First Two Weeks	Four to Ten Weeks
Infectious hepatitis	13	7	3
Infectious mononucleosis	3	2	0

all gave normal results. Four patients with biliary cirrhosis secondary to extrahepatic biliary tract obstruction all gave normal results but two of 18 with primary biliary cirrhosis were positive.

In the group with non-biliary cirrhosis, four of 14 with cirrhosis associated with alcoholism, 10 of 43 with cirrhosis of unknown aetiology, one of 11 in the 'juvenile' group, and two of four with haemochromatosis gave positive results. It was impossible to relate these findings with a previous history suggestive of infective hepatitis. Two of four patients with haemolytic anaemia and jaundice gave positive results, but all three cases with congenital hyperbilirubinaemia were negative. One of three cases with drug-induced jaundice was positive, and two of 18 with metastatic lesions in the liver were positive. Twenty-five patients with various collagen diseases were studied, six of whom gave positive results to titres of 1:80 or more; five of these had rheumatoid arthritis. One case of myocardial infarction was studied and was positive to 1:80.

A correlation was sought between haemagglutination titres and the various tests of liver function,

TABLE II

RESULTS

Condition	Total	0	<1:80	1:80	1:160	1:320	1:1,280
Normal	11	10	—	—	1	—	—
Infectious hepatitis	58	23	14	7	12	1	1
Infectious mononucleosis	8	2	3	—	3	—	—
Acute obstructive jaundice	12	10	2	—	—	—	—
<i>Non-biliary cirrhosis</i>							
Alcoholic	14	9	1	4	—	—	—
Non-alcoholic	43	26	7	5	2	3	—
'Juvenile'	11	7	3	1	—	—	—
Haemochromatosis	4	2	—	1	1	—	—
<i>Biliary cirrhosis</i>							
Primary	18	13	3	1	1	—	—
Secondary	4	3	1	—	—	—	—
Congenital hyperbilirubinaemia	3	2	1	—	—	—	—
Miscellaneous hepatic ¹	35	22	7	6	—	—	—
'Collagen' diseases ²	25	18	1	4	2	—	—
Non-hepatic miscellaneous ³	6	3	2	1	—	—	—

¹This group was composed of five patients with extrahepatic portal vein block, three with drug jaundice, two with amoebiasis, four with haemolytic jaundice, one with hydatid disease, one with Wilson's disease, one with chlonorchis sinensis, and 18 with metastatic deposits in the liver.

²This group was composed of 19 cases of rheumatoid arthritis, three of systemic lupus erythematosus, and three of ankylosing spondylitis.

³This group was composed of one case of coronary thrombus, one of pernicious anaemia, two with nephrotic syndromes, one with pustular psoriasis, and one with chickenpox.

including serum bilirubin, alkaline phosphatase, flocculation tests, and serum glutamic oxaloacetic transaminase. No correlation could be found with any one of these tests.

DISCUSSION

The results presented here confirm the findings of Havens (1958, 1960) that the sera of some patients with virus hepatitis contain a haemagglutinin for day-old chick erythrocytes. This agent is also found in some cases of infective mononucleosis. Havens, however, obtained positive results in 70% of 95 patients with infective hepatitis compared with 36% in the present study. On the other hand, we obtained positive results in 60% of our cases when sera were obtained within 14 days of the onset of symptoms, and this gives a better comparison with Havens' results, as the sera he used were obtained within 17 days of the onset of infective hepatitis.

Titres of 1 in 160 were found in three of eight patients with infective mononucleosis having a positive Paul-Bunnell test. This reflects the possible close relationship between the viruses of infective hepatitis and infective mononucleosis; in several cases a differentiation could not be made clinically, and depended upon the result of a Paul-Bunnell test.

Havens (1958, 1960) found that haemagglutinins persisted for many weeks into convalescence in about 50% of patients with infective hepatitis who had positive titres in the acute phase. Morris and Nakamura (1960) divided their cases of infective hepatitis into three groups according to their serial haemagglutination reactions. The most frequent was a high titre in the acute illness, falling to lower undetectable levels in convalescence. Next frequent were high haemagglutinin titres persisting throughout the course of the illness into convalescence, and the remaining patients showed no haemagglutinating activity at any time. The present study confirms the variable and unpredictable results obtained on serial examination and shows that, in two cases, the highest values were recorded during the third and sixth weeks respectively after the onset of symptoms.

Havens (1958) found positive titres in only 7% of normal patients, 17% in patients with other acute and chronic disease, and 12% in patients with chronic hepatic disease. In the present series, positive results

were obtained in a much higher proportion of patients, for example, in 23% of 43 patients with non-alcoholic portal cirrhosis, and in four of 14 with cirrhosis of the alcoholic. Five of 19 patients with rheumatoid arthritis gave positive results, two to titres of 1:160.

The present study confirms Havens' finding that obstructive jaundice is associated with a consistent failure to detect the haemagglutination reaction. No positive results were found in cases of extrahepatic biliary obstruction and in only two of 18 cases of primary biliary cirrhosis. It also confirms Havens' failure to find a correlation between haemagglutination titres and any specific test of liver function. Of special interest is the lack of correlation with the serum oxalo-acetic transaminase level.

Control studies showed that the haemagglutination titres obtained in the present study are comparable with those of Havens (Table I). This suggests that the different results obtained in various disease conditions may be due to factors other than technical ones. The lower incidence of positive titres in infective hepatitis would lend support to the view that several distinct viral agents may be responsible for this clinical condition. The higher incidence of false positives in other conditions is difficult to explain, and casts serious doubts on the value of the method in specific diagnosis. It is of some value, however, in the differential diagnosis of a jaundiced patient, for if a positive result is obtained within the first 14 days of the onset of symptoms an obstructive cause is excluded.

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