Havens' haemagglutination test in infective hepatitis¹

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In an attempt to find a diagnostic serological test in hepatitis, Havens in 1958 reported the results of his studies using red cells from day-old chicks. He showed that positive haemagglutination tests were found in the sera from 70% of patients in the early stages of viral hepatitis (Havens, 1959, 1960). The exact mechanism of this reaction is not known. Turner, Jha, Crowley, and Sherlock (1962), using the same test, found positive titres in 60% of sera from patients within two weeks of the onset of symptoms of hepatitis. We have used this procedure while investigating an epidemic of infective hepatitis in Bristol; the epidemiological details of this outbreak have been reported elsewhere (Bothwell, Martin, Macara, Skone, and Wofinden, 1963). We report the results obtained during the early phase of the disease and subsequently over a five-year follow-up period.

MATERIAL

Initially, sera were collected from 533 patients; all were jaundiced during the attack, and 73 sera were obtained within the first four weeks of the illness. The test was repeated on 203 subsequent sera. The relationship between the onset of the disease and the times at which the sera were obtained is shown in Tables I and II; sera were stored at -20°C until the tests were performed.

METHOD

HAEMAGGLUTINATION REACTION Havens' method (Havens, 1958) was used without modification.

A negative result was recorded when there was (1) a button of cells with a smooth margin, or (2) a ring of cells with smooth inner and outer edges in the tubes.

A positive result was recorded when agglutination was seen at a dilution of 1 in 80 or greater; positive results were assessed as + + + +, + + +, and ±. For the sake of simplicity only these three positive grades were employed.

When a doubtful result was seen in a control tube, the batch and the control were repeated.

LIVER BIOPSIES In nine patients whose sera showed persis-

tent strongly positive haemagglutination titres and protein alterations, aspiration liver biopsies were performed.

RESULTS

INITIAL HAEMAGGLUTINATIONS The positive and negative titres which were obtained are shown in Table I. Of the 533 sera tested, 143 (26.8%) were positive. These positive results were obtained in 49.3% of sera from patients who were within the first four weeks of the attack; in 25.6% of those from two to six months after the onset; and in 17.2% of sera obtained during the next six months. Positive results were also found in 25% of the sera which were obtained from one to two years after the onset.

TABLE I

Time after Onset when Sera Obtained (mth)	Total No. of Sera	Total Positive	Positive			Total Negative
			++++	+++	±	
Within one	73	36 49.3%	20	11	5	37
2 to 6	176	45 25.6%	22	14	9	131
7 to 12	122	21 17.2%	8	6	7	101
13 to 24	157	40 25%	21	7	12	117
25 to 36	5	1	1	—	—	4
Totals	533	143	72	38	33	390

In Table II the results of the tests which were repeated in 203 sera are shown. Of these, positive titres were seen in 59 (29%), 10 of which were still positive three to five years after infective hepatitis. Of 17 sera in which negative results were obtained initially, there was an alteration to definite positive titres later on during the patients' convalescence. On the other hand, 41 initially positive became negative, and 103 sera showed consistently negative results.

There was no relationship between a positive test and a raised ESR (>20 mm in 1 hr, Westergren). High ESRs occurred in 63% of patients tested

¹This work formed part of a thesis submitted by one of us (B.A.) for the degree of Doctor of Medicine in the University of Bristol.

TABLE II

REPEAT HAEMAGGLUTINATIONS IN INFECTIVE HEPATITIS

Time after Onset when Sera Obtained (mth)	Total No. Tested	Total Positive	Total Negative
First 6	35	18	17
7 to 12	27	12	15
13 to 24	65	11	54
25 to 36	59	8	51
37 to 48	11	5	6
49 to 60	6	5	1
Totals	203	59	144

Positives

First positives that became more positive	= 19
First negatives that became positive	= 17
First positives that remained the same	= 13
First positives that became less positive	= 10

Negatives

First positives that became negative	= 41
First negatives that remained negative	= 103

within four weeks of onset and in from 18 to 29% tested in periods from 12 to 42 months after the onset. Neither was there a relationship between a positive test and abnormal serum proteins, though 79% of those with a positive test had raised serum α_2 γ or β globulin levels.

LIVER BIOPSIES The histological studies did not show any abnormality.

DISCUSSION

A review of the available literature on the haemagglutination test shows that none of the published reports was based on a single epidemic from one city. The work reported has been done mainly on patients with viral hepatitis in military hospitals and some from scattered civilian institutions.

Of our total 73 sera from patients during the first four weeks of the attack, positive titres were found in 49.3%, whereas Iber (1964) over an exactly comparable period obtained only 31% positive results in 64 sera. Turner *et al* (1962) reported 60% positives in 58 sera from patients within two weeks of the onset of symptoms; Havens (1960) tested 95 sera obtained during the first 17 days after the onset of infective hepatitis and found that 70% of these were positive. These results thus compare on the whole with our figures during the survey of an epidemic.

During the remainder of the first six months we tested 217 sera and in 29.8% obtained positive results. The only comparable data are those of Iber (1964). He found 15.9% positive in his 132 cases tested at this time. There are no reports of the test having been done after six months from the onset of the disease.

The mechanism of the haemagglutination is unknown; any evidence of a direct relationship with circulating virus still awaits confirmation. The factor responsible for a persistently positive haemagglutination reaction is also not known. There is no obvious relationship with serum protein abnormalities, although a large number of our patients had abnormal serum proteins for several months, some up to five years, after the attack. This is of interest and may be of practical importance, because abnormal serum proteins and a raised ESR may be mistakenly attributed to continuing liver disease. A gamma globulin has been suggested as the cause of the positive haemagglutination by Havens (1962). A raised gamma globulin level in our series was not related to a continuing positive test, but immuno-electrophoresis may possibly have been more discriminating. Although a positive haemagglutination reaction could indicate a continuing hepatic disorder, our nine liver biopsies have shown no persisting damage.

There was no apparent connexion with the clinical severity of the attack of infective hepatitis. Havens (1962) suggests that the appearance of haemagglutinins is intimately related to the hepatitis and consequently their diminution or disappearance from the blood is usually associated with early recovery. However, persistent positive haemagglutinins were seen in our series in patients who had all apparently recovered completely.

Iber found that this test was completely non-specific; he suggested that it was of no value in determining the cause of liver disease. He obtained positive results in cirrhosis, alcoholic liver disease, nephrosis, and heart failure (Iber, 1964). Our study shows that the test may remain positive without any apparent persistent liver disease. The test is time consuming and does not differentiate infective hepatitis from other types of liver cell jaundice. The cause of its persistence is of interest but further detracts from its usefulness.

SUMMARY

Havens' haemagglutination test was performed on 533 sera from patients during an epidemic of infective hepatitis in Bristol, and was repeated in 203 of these cases. Positive tests were found in 49% of the patients within the first four weeks of the attack, and in a few as long as five years later.

Persistent abnormal levels of serum protein were also found; however, the positive haemagglutination tests were not related to these abnormalities.

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