Reaction of Viral Hepatitis Sera with *M. rhesus* Erythrocytes.* (22814)

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Latent or asymptomatic hepatic disease has been demonstrated in apparently healthy blood donors, and has been shown to be transmissible(1). In a recent study(2) 3655 apparently healthy blood donors were given a series of hepatic tests. According to the authors' evaluation 29% of these showed abnormalities, 17% "moderate" and 12% "severe." The authors concluded there was evidence that some, but not all, asymptomatic carriers will be excluded by the "hepatic test" screen, but that elimination of so large a fraction of blood donors would create a serious problem in supplying donor blood. It is generally agreed that some type of screening is needed, and that absence of jaundice during a number of months preceding blood donation does not insure freedom from infectivity.

Heterospecific serological tests have been found very helpful in the laboratory study of infectious diseases; agglutination of sheep erythrocytes in serum from patients with infectious mononucleosis has become a definitive test in diagnosing this condition. This suggested the possibility that a similar test might be found which would aid in the detection of viral hepatitis. Accordingly, sera from individuals diagnosed as having viral hepatitis were tested for their ability to agglutinate red cells of mouse, sheep, dog, guinea pig, steer, chicken, rabbit, horse, and So. American ring-tailed monkey, with no evidence that the cells could yield useful information. However, when red cells of rhesus monkey were used, agglutination was observed to a degree which was noticeably greater than was the sera from random controls. Erythrocytes from this species were therefore chosen for more intensive study.

Methods. *Macacus rhesus* red blood cells were collected in A. C. D. solution and stored in the refrigerator. A working suspension was prepared by washing the cells 3 times in 0.9% NaCl solution, resuspending them after the last washing to make up a 2% suspension by volume. The working suspension is made up just before using, and unused portions are discarded. The cells stored in A. C. D. solution gradually lose their sensitivity and should not be used after 10 days' storage. Sera to be tested were inactivated at 56C° for 30 minutes. 0.2 ml of serum was added to 0.2 ml of 0.9% NaCl solution and mixed; 0.2 ml of this dilution was transferred serially through 6 tubes, each containing 0.2 ml of diluent, giving a series of dilutions ranging from 1:2 to 1:64. 0.2 ml of cell suspension was added to each tube and the set was incubated for 1 hour at 37°C. Cells were sedimented by slow centrifugation (1000 r.p.m. for 2 min.) and degree of agglutination was estimated after gentle resuspension. The end-point was considered to be the tube containing the highest dilution of serum in which a definite agglutination was macroscopically visible. One of the problems in this kind of study is selection of a control group which may be presumed to be free from false positive reactions. In this case we chose sera from the pilot tubes of bank blood, since donors are questioned as to possible attacks of jaundice in the past, and must conform to certain physical standards indicating their apparent healthy condition. Even so, we must assume that some of these may be asymptomatic carriers of one of the hepatitis viruses, so the occurrence of positive reactions in this group cannot be considered as being false positives. In such a situation where control and experimental groups may overlap it is necessary to rely upon the differences in distribution of the results rather than upon absence of any positive reactions in the control group.

In addition to the control group, 3 others were studied. These included a group of patients diagnosed as having viral hepatitis,

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TABLE I. Agglutination Titers against Rhesus Erythrocytes.

Group	Highest serum dilution giving a positive test						
	Under 1:2	1:2	1:4	1:8	1:16	1:32	Total
I. Blood donors	65	18	27	12	3	0	125
II. Jaundiced, exclusive of viral hepatitis	8	4	2	0	0	0	14
III. Infectious mononucleosis	2	5	1	4	3	0	15
IV. Viral hepatitis	0	2	3	8	5	1	19

another group of jaundiced patients whose liver pathology was due to extrahepatic obstruction, Laennec's cirrhosis or malignancy, and a third group of individuals whose illness was diagnosed as infectious mononucleosis.

Results. The results of the agglutination tests are listed in Table I and presented graphically in Fig. 1. Table I shows that distribution of the titers in the second group (patients whose icterus was not due to viral hepatitis) did not differ significantly from the blood donor group. Therefore these two were combined in Fig. 1 to form the control group. Sera in this group were found to have comparatively low titers, 124 of the 139 specimens (89%) being less than 1:8. On the other hand, sera of the viral hepatitis group showed higher titers, 14 of the 19 specimens being positive in dilutions of 1:8 or more. The infectious mononucleosis sera were less uniformly distributed; the possible significance of this is discussed below. Table II compares the distribution of the titers observed in 19 hepatitis patients with the distribution which might have been predicted from the frequencies found in the control group.

Discussion. These observations indicate that one of the results of viral hepatitis is an increase in the ability of the patient's serum to agglutinate the red cells of the rhesus monkey. We are not able to say whether this effect is due to the presence of a true antibody, to the presence of the virus itself, or whether the reaction is based upon the appearance of an abnormal serum constituent resulting from physiological disturbances related to the disease. A test such as we have described, if proven to be reliable, should have two important functions. First, it would be a valuable aid in the differential diagnosis of the jaundiced patient. In our limited series the individuals whose jaundice was related to hepatic disease other than viral hepatitis fell into the normal category insofar as the rhesus erythrocyte agglutina-

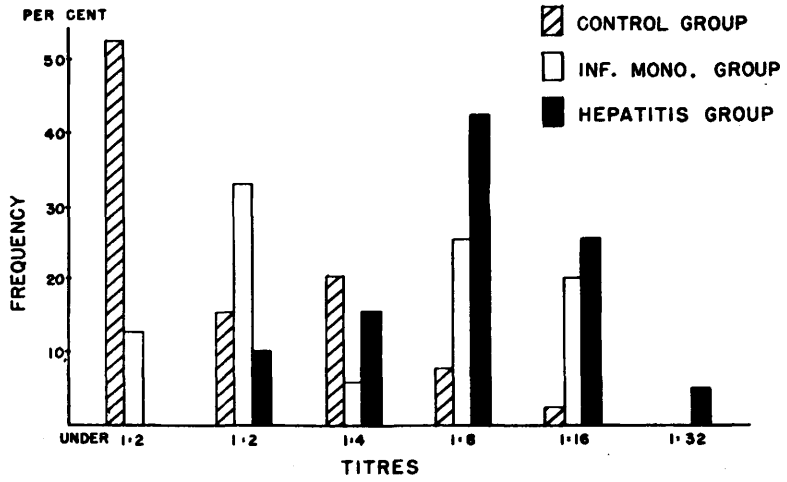

FIG. 1. Comparison of titres observed in control, infectious mononucleosis and viral hepatitis groups. The values represent the percentages of each group having the indicated titres.

TABLE II. Comparison of Expected and Observed Distribution of Titers in the Hepatitis Group.

	Titers					
	Under 1:2	1:2	1:4	1:8	1:16	1:32
Expected frequency based on control group	10	3.1	3.9	1.6	0.4	0
Observed frequency in hepatitis group	0	2	3	8	5	1

tion is concerned. Of the 19 cases of viral hepatitis, 14 showed elevated agglutinations titers. Thus, an agglutination titer of 1:8 or greater would support the diagnosis of viral hepatitis, while a titer less than 1:8 would argue against such a possibility. Since the test is positive (a titer of 1:8 or more) in almost one half of our cases of infectious mononucleosis. it cannot be said that it is a specific test for viral hepatitis. However, the liver is sometimes involved in infectious mononucleosis, so the positive tests obtained here may be related to hepatic disturbance. Furthermore, since infectious mononucleosis is ordinarily recognized by hematological and serological tests, the value of the rhesus erythrocyte agglutination reaction is not decreased by this finding.

A second possible field for use lies in the screening of donors to be used in the collection of whole blood or blood plasma. If the test were applied to the donors reported here, 15 of the 125 examined would have been rejected as possible asymptomatic carriers of viral hepatitis. Of course the evidence is purely presumptive, and further studies may show that such individuals may in fact not be carriers. However, since a substantial percentage of infected individuals in the viral hepatitis group (Gr. IV.) is detected by the test, its use as a screening device should be investigated. The fact that some infectious mononucleosis sera give positive reactions does not detract from its possible value as a screening procedure since it may be equally desirable to eliminate these individuals from the donor groups.

Summary. A hemagglutination test is described, wherein the sera of individuals diagnosed as having contracted viral hepatitis agglutinate the red blood cells of the rhesus monkey in dilutions of 1:8 or greater, as contrasted with the sera of a majority of apparently healthy blood donors and of jaundiced patients in whom a diagnosis of viral hepatitis was excluded. The observation, although made empirically and requiring confirmation, appears to offer promise and is being investigated at greater lengths.

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