semipurified inhibitor by isolation of the fast-moving, active component in the electrophoresis apparatus(10). A preparation with PF 190 was obtained from a starting material with PF 44. The yield of such highly purified inhibitor by this method is still smaller than that obtained through the use of electrophoretic precipitation (cited above).

In relation to these methods, the method based on filtration possesses the distinct advantages that (a) the procedure is extremely simple, (b) the conditions are mild, (c) no special apparatus is required, (d) the capacity is large, and (e) the products are superior in PF to all except those obtained by electrophoretic isolation; disadvantages are (a) the products are dilute and, for some uses, must be concentrated by pervaporation, with subsequent dialysis to reduce the salt concentration, and (b) the preparation of a batch of inhibitor requires at least 3 days, 2 for filtration and 1 for extraction. Thus far, attempts to reduce the time of filtration or extraction have proved unsuccessful.

The conditions affecting the filtration properties of the inhibitor have been studied extensively in experiments which will be reported separately.

Summary. A simple method is described for the purification of the egg-white inhibitor of influenza virus hemagglutination by filtration of diluted egg-white through rapid-flow filter papers and extraction of the unfilterable residue. The inhibitor has been purified about 80-fold in this way. The method has proved useful also for the further purification of acid-purified inhibitor. The advantages and disadvantages of the new method are discussed in relation to those of other methods.

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Failure to Find C-Reactive Protein in Viral Hepatitis.* (18116)

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The presence of C-reactive protein in the blood of patients with a wide variety of diseases has been described(1-4). Although the exact mechanism which potentiates the appearance of this substance is not clearly defined, it has been suggested that several different and apparently non-specific stimuli producing damage to tissues may be operative. Thus, C-reactive protein has been found in the blood of patients with infarction of the myocardium, acute and chronic infectious diseases, and following the intramuscular injection of such materials as vaccines and colloidal sulphur(5). Although frequent observations have been made of the appearance of C-reactive protein in the blood of patients with bacterial infections, only a limited amount of data is available concerning the presence of this substance in the blood of patients with viral diseases. It has been described(4) as occurring in occasional patients with influenza, viral hepatitis, infectious mononucleosis, poliomyelitis, mumps, and following vaccination for small-pox, but Löfström(3) reported that C-reactive protein was uncommonly present in the blood of patients

* This investigation was conducted with the aid of the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

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2. Ash, R., *J. Infect. Dis.*, 1933, v53, 89.

3. Löfström, G., *Acta med. Scandinav., Supplement 141*, 1943.

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5. Hedlund, P., *Acta med. Scandinav.*, 1944, v118, 329.

with viral infections unless secondarily invaded by bacteria.

During recent efforts to devise a diagnostic test for viral hepatitis, an apparently nonspecific serologic reaction was encountered(6). A large percentage of one group of patients with the disease had in their serums during the acute phase a substance which reacted with collodion particles in such a way that they were agglutinated by convalescent hepatitis gamma globulin. This substance was associated with the globulin fraction of the serum and, although the exact mechanism of the reaction was not defined, it was thought to be unrelated to hepatitis virus. In the attempt to elucidate the nature of this reaction, a large number of these serums were tested for the presence of C-reactive protein, and it is the purpose of this paper to report the results of these studies.

Methods and materials. Serums. Serums which were utilized in the tests were obtained from 90 patients with viral hepatitis in an American Army hospital in Germany. It is quite probable that many of these cases were examples of homologous serum hepatitis but, in view of the fact that there are no means of differentiating infectious hepatitis from serum hepatitis clinically, the cases have all been classified as viral hepatitis. Some of the serums were frozen at once and stored at a temperature of –4°C for periods ranging from one to 4 weeks. They were then shipped to the United States in containers with dry ice, and all serums were stored at dry ice box temperature for periods of one to 4 months before use. Other serums were tested within 2 hours after being obtained from the patients. For purposes of control, serums were obtained from patients with other acute infectious diseases including lobar pneumonia.

Technic of test. The test was performed according to the method of Abernethy and Francis(7), and dilutions of C-polysaccharide† ranging from 1:5000 to 1:640,000 were used.

Results and comment. The results of the determinations in the 90 patients are tabulated according to phase of disease in Table I. The uniformly negative reactions are slightly different from the results of Hedlund (4) who found 3 positive serums among 26 patients with viral hepatitis; 2 of these were obtained in the pre-icteric phase of disease. Almost half of the patients in the group described here were tested in the first 10 days of disease, and the large majority were examined within the first 2 weeks. Many of these serums contained greater than normal amounts of bilirubin, although a few of the earliest obtained had normal amounts of bilirubin.

TABLE I. Results of Tests for C-Reactive Protein in Serums of 90 Patients with Viral Hepatitis.

Days of disease	No. of serums Tested	Positive
5-7	7	0
8-10	33	0
11-14	32	0
15-21	9	0
22-28	4	0
29-63	5	0
Total	90	0

It is not known why C-reactive protein should be found so uncommonly in the blood of patients with viral hepatitis in contrast to its frequent appearance in the blood of patients with bacterial disease such as lobar pneumonia. It is possible that an insufficient number of patients were examined in the very early phase of disease. However, in view of the pathogenesis of viral hepatitis, it is of interest that such uniformly negative results were obtained if the mechanism of production of C-reactive protein depends, as has been hypothesized, entirely on the nonspecific destruction of tissue(8). Although hepatic necrosis occurs before the appearance of icterus in patients with viral hepatitis, it often continues into the second week of disease or even longer in certain patients. Occasional patients studied later in disease had active hepatitis. The fact that these tests were also negative is of particular interest in

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† This was kindly supplied by Dr. Colin M. MacLeod, New York University College of Medicine.

8. Löfström, G., *Brit. J. Exp. Path.*, 1944, v25, 21.

relation to the observations of Anderson and McCarty(9) on the value of the presence of C-reactive protein as an indicator of activity of disease in rheumatic fever.

The possibility must also be considered that the method of testing for C-reactive protein was not sufficiently sensitive to determine small amounts which might have been more readily detected by the use of C-protein antibody(10,11). However, the fact that the results were uniformly negative in contrast to the frequency of positive tests by the same method in various bacterial diseases raises the question as to whether this may be an expression of a fundamental difference in the pathogenesis of certain viral infections. It is noteworthy that most of the patients described here had an insidious onset of hepatitis with little or no fever. Although there is apparently no constant relationship between fever and the presence of C-protein in the blood, the association of fever, increase in alpha globulins and the presence of C-protein has been described in various other conditions. The alterations of serum proteins are of interest in this regard, and electrophoretic separation of serums containing C-reactive protein has revealed that it is associated with the alpha globulin fraction(12). It is known that this fraction is increased early in lobar pneumonia(13), while in viral hepatitis the beta and gamma globulins are more frequently increased, although the alpha fraction may also be augmented in occasional patients(14-16).

Summary. The serums of 90 patients in various phases of viral hepatitis were tested for the presence of C-reactive protein, and the results were uniformly negative. The significance of this is undetermined but the question is raised as to whether it may be an expression of a fundamental difference in the pathogenesis of certain viral infections.

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Production of Specific Antisera Against Sickle Cell Anemia Erythrocytes; Antibody in Sicklemia Sera.* (18117)

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A. *Production of specific rabbit antisera against sickle cell anemia erythrocytes.* During the course of experiments on the carbonic anhydrase content of erythrocytes from sickle cell anemia patients(1) it was noted that when such erythrocytes were diluted 1:100 or 1:200 with water, marked cloudiness appeared, in sharp contrast to the clear appearance of the same dilutions of normal erythrocytes. Similar but generally less marked clouding was often noted with erythrocytes from individuals with sickle cell trait. The well known resistance of sickling erythrocytes to hemolysis in hypotonic solutions was con-

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