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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1. Schneider, R. G., Levin, W. C. and Haggard, M. E., *J. Lab. and Clin. Med.*, 1949, v34, 1249.

sidered the possible explanation of the cloudiness, but microscopic examination of the centrifuged sediment revealed only amorphous material and no intact cells. Furthermore, the cloudiness did not disappear after prolonged standing, as would be expected with delayed hemolysis. When the sediment was centrifuged, washed repeatedly with water and analyzed, the nitrogen content was much higher than would be expected from the protein content of the stroma, after correction for the adsorbed hemoglobin(2). It was believed, therefore, that a protein was probably responsible for the clouding.

An attempt was made to determine whether sickle cell anemia erythrocytes are distinguishable immunologically from normal erythrocytes.

Methods. Erythrocytes from oxalated blood were washed three or four times in isotonic saline solution. After the final washing the cells were diluted with isotonic saline containing 0.12% of dextrose-sodium-citrate-citric-acid solution, so that 1.0 ml of the final suspension contained 0.1 ml of packed cells. When suspensions were used for more than three injections, a drop of 1:1000 aqueous merthiolate was added as preservative. Six rabbits were used for injection: 4 received erythrocytes from sickle cell anemia patient O.W. (OMNRh+), and 2 rabbits erythrocytes from sickle cell anemia patient J.A.G. (BNRh+). The animals were injected intravenously with 0.1 ml of cell suspension for 3 consecutive days. After a 4 day rest period they were reinjected for 3 consecutive days, and again allowed to rest for 4 days. Seven to 10 days after the fourth course of injections, the animals were bled. Thereafter they received a booster injection at approximately monthly intervals and sera were collected 7 days after each injection. The sera were inactivated at 56°C for 30 minutes, diluted 1:20, and absorbed. Absorptions were performed by adding to the diluted serum half the volume of washed packed erythrocytes of the appropriate blood group and type. The mixture was allowed to remain at room temperature 30 minutes, the cells centrifuged off, and the process repeated. Generally, 2 absorptions were found to be sufficient, although occasionally a third was necessary. The sera were stored in small vaccine bottles in a frozen state. In most experiments no preservative was added. This necessitated careful attention to the possibility of contamination and consequent development of non-specific agglutinins. Towards the end of this study sodium azide was added to the thawed sera to make a concentration of 0.1%, as has been suggested for the preservation of Rh antisera(3). Agglutination tests were performed by mixing 0.05 ml of serum with 0.05 ml of a 2% suspension of washed red cells. The tubes were allowed to stand at room temperature for one to 3 hours, and readings made under a dissecting microscope. The tubes were placed in the refrigerator overnight, after which final readings were made. Clumps visible to the naked eye were graded 2 to 3+. Clumps visible only under the dissecting microscope were graded 1+. In doubtful cases the tubes were centrifuged at about 750 r.p.m. for 1½ minutes before the final reading. An occasional cold agglutinin appeared after refrigeration, so that all tubes were allowed to come to room temperature before reading. Results were found more dependable when the erythrocytes were obtained from freshly drawn blood.

All individuals with the diagnosis of sickle cell anemia had been thoroughly studied in this hospital. Individuals with sickle cell trait were obtained by means of sickling surveys made periodically on all Negro patients. An effort was made to obtain test cells before patients had undergone transfusion, but this was not always possible, particularly among the individuals with sickle cell trait, some of whom had been admitted for surgery. Control erythrocytes were usually obtained from the blood bank. Most of the control cells were from white donors, but thirteen were from Negroes (not tested for the presence of sickling).

2. Schneider, R. G., and Levin, W. C., unpublished data.

3. Batson, H. C., Jayne, M., and Brown, M., *J. Lab. and Clin. Med.*, 1950, v35, 297.

Results. Of the 6 animals injected, 5 produced usable sera. Most of the tests were performed with sera from rabbits 319 and 320, injected with cells from patient J.A.G. (BNRh+) and from rabbit 323, injected with cells from patient O.W. (OMNRh+).

Table I shows the results of agglutination reactions with these sera against cells from 124 normal individuals, 19 individuals with sickle cell anemia, and 21 with sickle cell trait. Only those erythrocytes from patients with sickle cell anemia were agglutinated by the sera. None of the erythrocyte suspensions from individuals with sickle cell trait reacted with the sera. Two most likely explanations of this finding were considered: (1) That this was due to insufficient antibody in the sera to produce visible agglutination with the trait cell; (2) that the antibody was specific for sickle cell anemia erythrocytes. In order to test these explanations, absorption experiments were performed with cells from individuals with sickle cell trait and from those with sickle cell anemia. To the sera, absorbed as described previously, was added one-half the volume of washed, packed erythrocytes from individuals with the trait or the anemia. After standing 30 minutes at room temperature, the tubes were centrifuged, the serum drawn off, and the process repeated.

TABLE I. Summary of Agglutination Reactions of Anti-Sickle Cell Anemia Rabbit Sera with Erythrocytes of Normal Individuals and Those with Sickle Cell Anemia and Sickle Cell Trait.

Serum from rabbit No.	No. of individuals tested	Diagnosis	% positive
323	82	Normal	0
	17	Sickle cell trait*	0
	16	" " anemia	100
320	59	Normal	0
	13	Sickle cell trait	0
	19	" " anemia	100
319	100	Normal	0
	15	Sickle cell trait	0
	19	" " anemia	100
All three sera	124	Normal	0
	21	Sickle cell trait	0
	19	" " anemia	100

* Two of these patients were originally diagnosed as sickle cell anemia but re-evaluation of the clinical data makes the diagnosis of sickle cell trait more likely. A more detailed report of these patients will be made at a later date.

TABLE II. Absorption of Anti-Sickle Cell Anemia Rabbit Sera with Erythrocytes from Individuals with Sickle Cell Trait.

Date of absorption	Serum No.	Agglutination reaction with erythrocytes of patients with sickle cell anemia after absorption of sera with erythrocytes of patients with sickle cell trait
3/23	320	++
5/8	323	+
5/24	323	+
		+
		—
5/12	323	++
		+
		+
5/29	323	++
		++
		+

TABLE III. Absorption of Anti-Sickle Cell Anemia Rabbit Sera with Erythrocytes from Individuals with Sickle Cell Anemia.

Date of absorption	Serum No.	Agglutination reaction with erythrocytes of patients with sickle cell anemia after absorption of sera with erythrocytes of patients with sickle cell anemia
3/23	320	—
		—
		—
		—
3/28	319	—
		—
5/24	323	—
		—
		—

Table II demonstrates that absorption of sera with cells from sickle cell trait does not remove the agglutinins for sickle cell anemia erythrocytes. Of the eleven sickle cell anemia cell suspensions tested against various sera absorbed with sickle cell trait erythrocytes only one reaction (C.W.) was negative. This might well be explained by non-specific absorption.

Table III demonstrates that absorption of the sera with erythrocytes from individuals with sickle cell anemia completely removes all agglutinins in nine tests with erythrocyte suspensions of sickle cell anemia patients. It appears, therefore, that the anti-sickle cell

TABLE IV. Titrations of Anti-Sickle Cell Anemia Rabbit Sera 319, 320, 323 Against Erythrocytes from Individuals with Sickle Cell Anemia.

Dilutions × 20	Patient P.R.	Patient S.S.	Patient O.W.	Patient D.G.J.	Patient C.W.
Serum 319					
und.	+	+	++	+	+
2	+	—	++	+	+
4	+	—	++	—	+
8	—	—	+	—	—
16	—	—	+	—	—
32	—	—	—	—	—
64	—	—	—	—	—
128	—	—	—	—	—
Serum 320					
und.	+	+	++	+	+
2	+	+	++	+	+
4	+	+	+	—	—
8	+	+	+	—	—
16	—	+	±	—	—
32	—	—	—	—	—
64	—	—	—	—	—
128	—	—	—	—	—
Serum 323					
und.	+		+	++	+
2	+		++	+	+
4	+		++	+	+
8	+		+	±	—
16	±		±	—	—
32	—		—	—	—
64	—		—	—	—
128	—		—	—	—

anemia serum contains an antibody specific for sickle cell anemia erythrocytes.

Titrations of rabbit sera 319, 320, and 323 against erythrocytes from several sickle cell anemia patients are given in Table IV. The most frequently observed titer is 1-16 (times 20). The lowest is serum 319 against patient, S.S., positive only when undiluted (actually 1:20).

B. *Demonstration of agglutinins in sickle cell anemia and sickle cell trait.* An attempt was made to determine whether an agglutinin exists in the sera of individuals with sickle cell anemia and sickle cell trait.

Methods. Erythrocytes from oxalated blood were washed 3 to 4 times with isotonic saline. One-tenth milliliter of a 2% suspension of cells, either in saline or in 20% bovine albumin (Armour) was mixed with 0.1 ml of the patient's serum. Tubes were placed in the water bath at 38° for one hour, then centrifuged at 750 r.p.m. for 1½ minutes. Readings were made as described previously. Tests were also made to determine the presence of antibody (human globulin) absorbed to the erythrocytes, according to the method of Coombs, Mourant, and Race(4). The "direct" Coombs test was used, and final readings were made after refrigeration for 18 hours.

Results of agglutinin tests on the sera of 13 patients with sickle cell anemia are shown in Table V. Saline or albumin agglutinins are present in the sera of all but one of these 13 patients. All gave evidence of the presence of antibody adsorbed on the erythrocytes, by a positive reaction with the antiglobulin (Coombs) test.† Of the 11 individuals with sickle cell trait, 3 show saline agglutinins, four show agglutinins in albumin, while 2 have doubtful albumin agglutinins. All of our sickle cell anemia patients had received numerous transfusions. Some of the patients with sickle cell trait had been transfused for unrelated diseases. The possibility that agglutinin formation was related to repeated transfusions was considered. Accordingly, patients with no evidence of sickling, but who had had numerous transfusions were also examined for the presence of agglutinins. To date, six such patients have been tested for the presence of agglutinin. All have been negative.

Investigations are under way to determine whether the specific antigen of the sickle cell anemia erythrocyte is contained in the stroma or in the hemoglobin. The fact that it appears to be specific for sickle cell anemia would indicate that it is not related to the abnormal hemoglobin found by Pauling(5) to be present in erythrocytes with the sickling capacity. Pauling describes a quantitative difference between erythrocytes from individuals with sickle cell anemia and those with sickle cell trait, in that the former contain 100% of the abnormal hemoglobin, while the latter contain a mixture of about 50% of

4. Coombs, R. R. A., Mourant, A. E., and Race, R. R., *Brit. J. Exper. Path.*, 1945, v26, 255.

† We are grateful to Dr. J. M. Hill and Dr. Sol Haberman, Baylor Hospital, Dallas, Texas, for supplying some of the antiglobulin serum.

5. Pauling L., Itano, H. A., Singer, S. J. and Wells, I. C., *Science*, 1949, v110, 543.

TABLE V. Agglutinins in Sera of Individuals with Sickle Cell Anemia and Sickle Cell Trait.

Erythrocytes of:	Diagnosis*	Saline suspensions	Albumin suspensions	Antiglobulin (Coombs) test
W.B.	SCA	—	—	+
P.R.	"	+	+	+
N.N.	"	++		+
J.W.	"	+		+
A.B.	"	++	+	++
D.W.	"	—	++	+
S.S.	"	++	++++	+
D.G.J.	"	±	+	+
M.S.	"	+	+	+
W.K.	"	±	+	+
W.E.	"	—	++	
C.W.	"	—	++	+
O.W.	"	—	+	+
I.C.G.	T	+	++	+
M.B.	T	+	++	+
W.M.	T	—	—	—
G.E.	T	+	+	±
O.W.	T	—	—	—
A.S.	T	—	—	
C.M.	T	—	—	—
F.T.	T	—	±	±
D.K.	T	—	—	
C.J.	T	—	±	
S.J.	T	—	+	

* SCA indicates sickle cell anemia.
T " " " trait.

the abnormal hemoglobin and about 50% normal hemoglobin. Our results would indicate that there is a *qualitative* difference in the antigenic structure of erythrocytes from individuals with sickle cell trait and those with sickle cell anemia.

The demonstration of agglutinins in the sera of all the thirteen sickle cell anemia patients tested suggests that the hemolytic process in sickle cell anemia may be related to the presence of agglutinin in the patients' sera. Further experiments are in progress to determine the nature of this agglutinin and the significance of its presence in about one-third of our cases of sickle cell trait. Since our rabbit sera have given absolute differentiation between the sickling trait and the anemia, we believe that anti-sickle cell anemia rabbit sera may offer a diagnostic aid in the differentiation of sickle cell anemia from sickle cell trait in those cases in which the latter is complicated by other forms of anemia, and presents a difficult diagnostic problem.

Summary. 1. Erythrocytes from 2 individuals with sickle cell anemia, when injected into rabbits, produced antisera which agglutinated erythrocytes of 19 individuals with sickle cell anemia, but not those of 21 individuals with sickle cell trait nor those of 124 normal individuals.

2. Such sera may be useful in the differential diagnosis between sickle cell anemia and sickle cell trait.

3. The presence of agglutinin antibody has been demonstrated in the sera of all of 13 patients with sickle cell anemia. This finding may explain the hemolysis which is characteristic of this disease.

4. Of 11 patients with sickle cell trait, definite agglutinin antibody was demonstrated in the sera of 4, and doubtful agglutinin antibody in 2.

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Effects of HCl on Alkaline Phosphatase in Kidney and Intestine: Histochemical and Quantitative Study.* (18118)

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Numerous histochemical and biochemical studies have demonstrated the widespread distribution and specific location of alkaline phosphatase in various tissues. However, the

* An abstract covering part of this data appears in *Anat. Rec.*, 1949, v103, 29.

1. Bodansky, O., *J. Biol. Chem.*, 1937, v118, 341.
2. Cloetens, R., *Enzymologia*, 1939, v6, 46.