The resulting protein solution was found to have many properties of an antiserum specific to the phenylarsonic acid group. It gave precipitates with multihaptenic dyes and azoproteins containing this group, and not with other dyes or azoproteins, and the specific precipitates were dissolved by excess of dyes or haptenes containing this group but not by other dyes or haptenes, such as those containing the phenylsulfonic acid group.

A solution of 1 per cent. pneumococcus polysaccharide type III and 1 per cent. bovine γ-globulin was similarly held at 57° for 14 days. Some precipitate formed, which was removed. The resultant solution was found to precipitate type III polysaccharide but not types I or VIII (cross-reaction with type VIII was thus not shown) and to agglutinate pneumococci type III but not types I or II. Purified antibody solutions were made by adding 15 per cent. salt and bringing to pH 4 or adding 15 per cent. salt, calcium chloride, and calcium hydroxide to pH 8, thus precipitating polysaccharide and some protein. Each of the two solutions, after dialysis against 1 per cent. salt solution, was found to precipitate type III polysaccharide but not types I or VIII and to agglutinate type III pneumococci but not types I or II. Mouse protection tests and swelling tests have not yet been carried out.

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Our experiments are being continued.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF THE COMPLEMENT FIXATION TEST IN ROCKY MOUNTAIN SPOTTED FEVER

THE clinical differentiation between typhus fever and Rocky Mountain spotted fever may be difficult, especially when both diseases occur in the same region. The laboratory diagnosis may be made by the isolation of the virus from the patient, but this can only be satisfactorily performed if guinea pigs are inoculated immediately upon withdrawal of the blood. The agglutination test, using either typhus or Rocky Mountain spotted fever Rickettsiae is not satisfactory, while the Weil-Felix agglutination with Proteus OX 19 may indicate the presence of a Rickettsial disease, but a differentiation between typhus and Rocky Mountain spotted fever can not be made. It is for these reasons that a specific complement fixation reaction may be of value in distinguishing these two diseases.

Castaneda[1] prepared an antigen from the peritoneal washings of x-rayed rats inoculated with endemic Rickettsiae and reported positive complement fixation in typhus fever. Bengtson[2] described a specific complement fixation reaction for "Q" fever and endemic typhus, using an antigen prepared with Rickettsiae grown in the yolk sac of developing chick embryos (Cox method).[3] No satisfactory antigen for Rocky Mountain spotted fever had previously been prepared and it is the purpose of this communication to describe a specific complement fixation test which may be used in this disease.

The sera used in the tests were obtained from recovered human cases of Rocky Mountain spotted fever, "Q" fever and Brill's disease and from guinea pigs recovered from infections with Rocky Mountain spotted fever, endemic typhus, European typhus and "Q" fever. As controls, human sera were obtained from several different febrile diseases, strongly Wassermann positive sera, as well as normal sera. Likewise, normal guinea pig sera were used.

Two antigens, which were equally satisfactory, were prepared from tissue cultures grown by the semi-solid agar method in Kolle flasks.[4,5] The cultures were initiated with infected guinea pig spleen and grown in contact with ten-day chick embryo cells. Transplants were made every seven days by scraping off the infected cells from the surface of the medium and grinding in a tube with alundum. This material was again brought in contact with normal chick embryo cells. After several transplants, the cultures became exceedingly rich, so that a considerable number of Rickettsiae were seen in every field. The antigens employed were made from the 25th to the 35th transplant. Five cubic centimeters of a 1:10,000 merthiolate solution in saline was used to wash off the cells from each Kolle flask. When a certain amount of material was obtained, the Rickettsiae were extracted in the following manner. One batch was ground up in the Waring Blender and then frozen and thawed five times. The other was shaken in a large Pyrex flask with glass beads. The Rickettsial suspensions were then centrifuged in an angle centrifuge at 1,500 r.p.m. for 30 minutes. The supernatant fluid was removed and centrifuged at 4,500 r.p.m. for one hour. The sediment was then resuspended in 0.85

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per cent. saline to one fifth of the original volume. The final preparation contained a heavy suspension of Rickettsiae.

The antigen was titrated with a known guinea pig serum as well as with two known human sera. A 4 plus fixation was obtained with dilutions of the antigen up to 1:6. The antigen was found to be anticomplementary in a dilution of 1:2. An antigen dilution of 1:4 was used in all tests.

The usual hemolytic system, consisting of sheep cells, guinea pig complement and rabbit anti-sheep cell amboceptor was employed. The amboceptor was diluted to contain 3 M.H.D. in 0.25 cc. Equal amounts of amboceptor dilution and a 3 per cent. suspension of sheep cells were mixed together. The fresh complement was titrated on the day of the test.

Sera were inactivated at 56° C. for 30 minutes. Serum dilutions were made ranging from 1:3 to 1:192 using 0.25 cc amounts of each dilution in the test. Complement was diluted to contain 2 full units in 0.5 cc. A suitable antigen dilution was added in 0.25 cc amounts. Fixation was carried out for 20 hours in the ice box (4° C.) following which 0.5 cc of sensitized sheep cells were added to each tube and incubated for 30 minutes at 37° C. One and two units of complement in the presence of 0.25 cc of antigen dilution were also set up to indicate the validity of the results by showing the actual amount of free complement at the time the hemolytic system was added. The results of the tests are shown in Table 1.

TABLE 1

Human sera	Date of illness	Disease	1:3	1:6	1:12	1:24	1:48	1:96	1:192	Serum control
RO	1941	R.S.F.	4	4	4	4	4	2	1	0
WE	1938	R.S.F.	4	4	4	3	2	0	0	0
PA	1940	R.S.F.	4	4	3	2	1	0	0	0
SH	1941	R.S.F.	4	4	4	4	4	3	2	0
SM	1940	R.S.F.	4	4	4	4	3	2	1	0
RA	1941	R.S.F.	4	4	4	4	4	2	1	0
HO	1941	R.S.F.	4	4	4	4	4	4	2	0
HU	1937	R.S.F.	4	4	3	2	–	–	–	0
LO	1941	R.S.F.	4	4	4	4	3	2	–	0
2 cases		Brill's	0	0	0	0	–	–	–	0
11 sera		Various febrile cases	0	0	0	0	–	–	–	0
8 sera		Wassermann positive	0	0	0	0	–	–	–	0
2 sera		"Q" fever	0	0	0	0	–	–	–	0
11 sera		Normal	0	0	0	0	–	–	–	0

We have examined the sera from nine cases of Rocky Mountain spotted fever and obtained positive complement fixation reactions in all. The oldest case (HU) had the disease 4½ years prior to the examination of the serum, while the most recent serum (LO) examined was obtained on the 12th day of illness. This latter finding is significant, for if in subsequent cases it can be shown that antibodies can be demonstrated so early in the disease the complement fixation test may prove to be a real aid in diagnosis. In three cases, (PA), (SH) and (SM), several specimens of serum were examined over a period of three and six months and no significant change in titre was noted.

There were two cases where the possible diagnosis of typhus fever was made. The subsequent course of the disease, a negative fixation test for typhus fever and a positive test for Rocky Mountain spotted fever indicated that we were dealing with the latter disease.

TABLE 2

Guinea pig sera	Disease	1:3	1:6	1:12	1:24	1:48	1:96	1:192	Serum control
No. 761	6 days after drop Temp.	0	0	0	0	–	–	–	0
" 743	12 days after drop Temp.	4	4	3	3	2	0	0	0
" 379	R.S.F.	4	4	4	4	3	1	0	0
" 489	R.S.F.	4	3	3	2	1	–	–	0
" 277	R.S.F.	3	3	2	1	1	–	–	0
" 220	R.S.F.	4	4	4	4	3	1	0	0
" 486	R.S.F.	4	4	4	4	3	2	0	0
" 498	R.S.F.	4	4	4	3	1	0	0	0
" 490	R.S.F.	3	2	2	1	–	–	–	0
7 sera	Endemic	0	0	0	0				0
6 sera	Epidemic	0	0	0	0				0
3 sera	"Q" fever	0	0	0	0				0
15 sera	Normal	0	0	0	0				0

In guinea pigs (Table 2), fixation was obtained with eight sera. It is of interest to note that one guinea pig gave a negative reaction six days after the return of temperature to normal, while another gave a positive test after twelve days. The occurrence of fixing antibodies during the course of the illness in guinea pigs and monkeys is now being studied. No fixation was obtained with sera from endemic typhus, epidemic typhus or "Q" fever.

The results obtained in the tests described indicate that the complement fixation test may be employed in diagnosing Rocky Mountain spotted fever.

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