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Complement Fixation in "Q" Fever.

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Agglutination tests in "Q" fever have been described by Burnet and Freeman,[1, 2, 3] by Smith, Brown and Derrick,[4] and by Bengtson.[5, 6] In the present work complement fixation in "Q" fever has been investigated to determine whether this test might also be of value in tests for diagnosis and immunity. Such a test has been previously described by Castaneda[7] for Mexican typhus.

Material and Methods. The sera employed were from recovered "Q" fever cases and from recovered infected guinea pigs. The human sera tested were from cases involved in a recent laboratory outbreak[8] and from normal individuals employed in the same building. The guinea pigs were animals used in the routine passage of the American and Australian "Q" fever strains, the American strain being the X-strain described by Dyer[9] and the Australian strain was the strain received from Dr. Burnet, referred to in the publication of Dyer.

Preparation of Antigens. Antigens were prepared from spleens of mice infected by the method of Burnet and Freeman and from yolk sacs infected by the method of Cox.[10] The mouse spleen material was from mice several passages removed from the first inoculation of mice with guinea pig virus, or from mice which had been inoculated with heavily infected yolk sac. These spleens contained numerous rickettsiae. The yolk sac material was from eggs in the 5th or 6th passage of the virus when innumerable rickettsiae were present.

The most suitable antigens were apparently obtained from the material showing the greatest number of rickettsiae. The mouse material was found to give very satisfactory results in the tests with

1-3 Burnet, F. M., and Freeman, M., *Med. J. Austral.*, 1937, **24**, 299; 1938, **25**, 296, 1114.

4 Smith, D. J. W., Brown, H. E., and Derrick, E. H., *Med. J. Austral.*, 1939, **26**, 13.

5-6 Bengtson, Ida A., *Pub. Health Rep.*, 1941, **56**, 272, 327.

7 Castaneda, M. R., *J. Immunol.*, 1936, **31**, 285.

8 Dyer, R. E., Topping, N. H., and Bengtson, I. A., *Pub. Health Rep.*, 1940, **43**, 1945.

9 Dyer, R. E., *Pub. Health Rep.*, 1938, **53**, 2277.

10 Cox, H. E., *Pub. Health Rep.*, 1938, **53**, 2241.

guinea pig serums but was not as satisfactory with human sera. The yolk sac antigens were therefore employed.

The method of preparation was as follows: The yolk sacs of 6 to 8 eggs were removed at the time when the embryos were beginning to die, usually about the 3rd day after inoculation. After draining for a few minutes the yolk sacs were macerated with sterile alundum and suspended in 0.85% sterile saline to a concentration of 10%. This was centrifuged lightly in the horizontal centrifuge to precipitate coarse particles. The supernatant fluid was then centrifuged in the angle centrifuge at 4000 r.p.m. for one hour. The precipitate was removed and suspended in 0.85% sterile saline to the original volume, the supernatant fluid being retained to test for presence of antigen. The suspension of rickettsiae was allowed to stand in the refrigerator for a week or longer, during which time more tissue precipitated, leaving the rickettsiae in suspension, with little tissue remaining. The Australian strain of "Q" fever was used in the experiments reported here.

A titration of the antigen prepared as described, for the complement fixing capacity, was carried out with a known positive guinea pig and human sera. Titrations were also made of the same antigen steamed for 30 minutes in the Arnold sterilizer, and of the supernatant fluid from the high speed centrifugation as well as a Berkefeld-N filtrate of the supernatant fluid.

A 4 plus fixation was obtained with dilutions of the rickettsial suspension up to 1/8. Heating in the Arnold sterilizer had the effect of reducing the results 100%, a 4 plus fixation being obtained with dilution 1/2 and 1/4, but not 1/8. No fixation whatever was obtained with the supernatant fluid from the high speed centrifugation or the Berkefeld filtrate of this fluid. The supernatant fluid and Berkefeld filtrates of the mouse spleen material on the other hand at times gave as good fixation as the suspension of the rickettsiae in the tests with guinea pig serum.

The Test. Guinea pig complement was titrated on the day of the test. The amboceptor was diluted to contain 2 units in 0.2 cc and equal amounts of amboceptor dilution and a 5% suspension of sheep's red blood cells were mixed. The sera to be tested were inactivated at 56°C for 30 minutes and dilutions prepared with sterile saline, ranging from 1/2 to 1/128. The serum dilutions in 0.2 cc amounts were mixed with 2 units of complement contained in 0.2 cc and with 0.2 cc of the antigen appropriately diluted. Serum controls without antigen were included using dilutions of 1/2 and 1/4 in all tests and in certain tests all dilutions up to 1/128. The mixtures

were incubated for 1 hour in a 37°C water bath and 0.4 cc of sensitized cells then added and incubation continued for another hour followed by overnight storage at refrigerator temperature. Controls on the hemolytic system, the antigen, and sensitized cells were included in each test.

Results. The results obtained in tests with 13 human sera from 10 individuals, including controls and 5 guinea pig sera are shown in Table I. The antigen was diluted ¼.

In Table II are shown the results of a test for specificity, using sera from guinea pigs recovered from endemic typhus, European typhus, and Rocky Mountain spotted fever. A serum from a recovered human endemic typhus case not shown in the table gave negative results.

Discussion. The test as carried out gave clear-cut results. Complement-fixing antibodies were present in both human and animal sera as early as 9 days after the beginning of fever in guinea pigs and 13 days after onset in man. The titer of the sera increased in 22 to 23 days and was still higher in the case of one of the human sera tested in 32 days when it reached a titer of 1/128. A titer of 1/32 was present in 285 and 305 days after onset in 2 of the human cases.

TABLE I.
Complement Fixation with Human and Guinea Pig Sera.

Sera	Days after onset	Dilutions of sera							Serum controls			
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:2	1:4	1:8	1:16
Human												
AKa*	13		1	4	4	2	0	0		0	0	0
AKb*	23		3	4	4	3	1	0		0	0	0
AKc	32	4	4	4	4	4	4	3	0	0		
RRa*	48		4	4	4	2	1	0		4	4	1
RRb	307	4	4	4	3	2	0	0	0	0		
LB	285	4	4	4	4	2	1	0	0	0		
CP	305	3	3	3	3	3	1	0	0	0		
5 Normal (?)†		2	1	0	0	0	0	0	0	0		
6 " "		3	3	3	0	0	0	0	0	0		
7 " "		4	3	3	1	0	0	0	0	0		
8 Normal		0	0	0	0	0	0	0	0	0		
9 "		0	0	0	0	0	0	0	0	0		
10 "		0	0	0	0	0	0	0	0	0		
Guinea pig												
1	9	3	3	3	2	1	0	0	0	0		
2	22	4	4	4	4	4	4	1	0	0		
3	31	4	4	4	4	4	4	1	0	0		
4	60	4	4	4	4	4	4	0	0	0		
5 Normal		0	0	0	0	0	0	0	0	0		

*Sera stored at ice box temperature for 1 month or longer which were treated by the Sachs[11] method to remove anticomplementary substances.

† Nos. 5 to 7, inclusive, from individuals engaged in studying "Q" fever.

[11] Sachs, H., *Berl. Klin. Wchnschr.*, 1921, **58**, 1075.

TABLE II.
Test for Specificity.

Infection	No. of guinea pig	Days after onset of fever	Dilutions of sera						Serum controls (No antigen)	
			1:2	1:4	1:8	1:16	1:32	1:64	1:2	1:4
Endemic typhus	1	10	0	0	0	0	0	0	0	0
	2	73	0	0	0	0	0	0	0	0
European typhus	3	10	0	0	0	0	0	0	0	0
	4	60	0	0	0	0	0	0	0	0
Rocky Mountain spotted fever	5	40	0	0	0	0	0	0	0	0
	6	105	0	0	0	0	0	0	0	0
"Q" fever	7	11	2	2	2	1	0	0	0	0
	8	44	4	4	4	4	4	2	0	0

The specificity of the test is indicated by the results obtained with sera from guinea pigs inoculated with European typhus, endemic typhus, and Rocky Mountain spotted fever, no fixation being present in dilutions as low at ½. The tests were made as early as 10 days following beginning of fever and as late as 105 days after.

The sensitivity of the test is indicated by results obtained with the sera of 3 workers who were engaged in the study of "Q" fever. None had suffered an apparent infection and it may be that a certain immunity was established in the handling of the infected material. On the other hand, all had received repeated inoculations of Rocky Mountain spotted fever vaccine prepared from infected ticks, and it is possible that certain lots of vaccine may have been made from ticks simultaneously infected with Rocky Mountain spotted fever and "Q" fever.

The superiority of the yolk sac antigen over the mouse spleen antigen in the tests on the human sera may be accounted for by the greater number of rickettsiae present, but it is also probable that the antigenic substance was more thoroughly separated from the suspension in the case of the yolk sac than the mouse spleen since, as has been pointed out, negative results were obtained with the supernatant fluid of the yolk sac material and positive results with the supernatant fluid of the mouse spleen material.

Summary. A complement-fixing reaction for "Q" fever which is specific among the rickettsial diseases is described. Yolk sac antigen was superior to mouse spleen antigen in tests with human sera. The test was shown to have a good degree of sensitivity and it appeared 9 to 13 days after onset of the disease and persisted for at least 305 days. The indications are that the test is of value for diagnosis and that it affords evidence of immunity.